BIOSYNTHESIS OF DRUGS

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BIOSYNTHESIS AND BIOGENESIS

Metabolism is the series of pathways operating when biological systems synthesize their constituents. *Biosynthesis* is the experimentally established pathway of formation of secondary metabolites; where experimental proof is absent, the term *biogenesis* is used.

SECONDARY METABOLITES

Reaction products that are necessary for the generative functions (respiration and catabolism) of an organism are termed primary metabolites; those resulting in products used for other functions are known as *secondary metabolites.* Such compounds typically characterize the individuality of an organism or a group of organisms; this study is termed chemotaxonomy. Consequently, the biosynthetic pathways of secondary metabolism are not random but are highly conserved. Thus, a given plant family may produce substantial numbers of a certain type of metabolite (e.g., quassinoids in the Simaroubaceae), whereas another family produces quite different metabolites (e.g., monoterpene indole alkaloids in the Apocynaceae).

THE PRECURSORS

A select group of primary metabolites, predominantly acetate, shikimic acid, isopentenyl pyrophosphate, and a few amino acids, is responsible for the diversity of the 135,000 plant-derived secondary metabolites in 12 major classes (Fig. 1). Only those classes with some social or economic significance as bioactive agents are discussed here.

SIGNIFICANCE OF BIOSYNTHESIS

Biosynthetic knowledge is an integral and essential aspect of natural products chemistry and has recently assumed high-profile academic and commercial significance for biotechnological reasons. It is the foundation permitting a systematic and rational framework for organizing the bewildering structural diversity of mammalian, arthropod, insect, plant, microbial, and marine secondary metabolites. It provides structural clues for new metabolites through biogenetic possibilities based on established biosynthetic schemes. It permits the bioengineering of metabolic pathways for enhanced yields or the altering of desired product profiles for greater economic gain. Manipulations at the genetic level may afford a substantially new array of metabolites for future drug discovery. Finally, it is of fundamental human curiosity to discern how secondary metabolites are produced in living systems. The modification of such processes at the enzyme or gene level may be of critical importance in the treatment of mammalian processes involved in disease states; cholesterol synthesis-inhibiting drugs, such as mevinolin, are an example.

METHODS IN BIOSYNTHESIS

Biogenetic theories arose as the need to classify diverse secondary metabolites became apparent. Biosynthetic experimentation tested hypotheses when the precursors became available in radio-, and subsequently stable, isotope labeled forms. It focuses on the study of precursor relationships (including the stereochemistry of specific processes) and of the enzymes involved in the succinct steps in the pathway. A typical experiment involves the administration of a potential precursor in labeled form to the organism, at a time when it is known that the organism is actively producing the metabolite of interest. After an appropriate period of time, the organism is processed for the metabolite of interest and the isotope content located and measured. Most of the fundamental pathways of natural product biosynthesis were established using the radioactive isotopes of hydrogen and carbon, ^{3}H and ^{14}C. Extensive use is now made of the stable isotopes of the key atoms present in natural products, ^{13}C, ^{18}O, ^{15}N, and ^{2}H.

Evaluation of the structural complexity of natural products, coupled with prior biosynthetic knowledge,

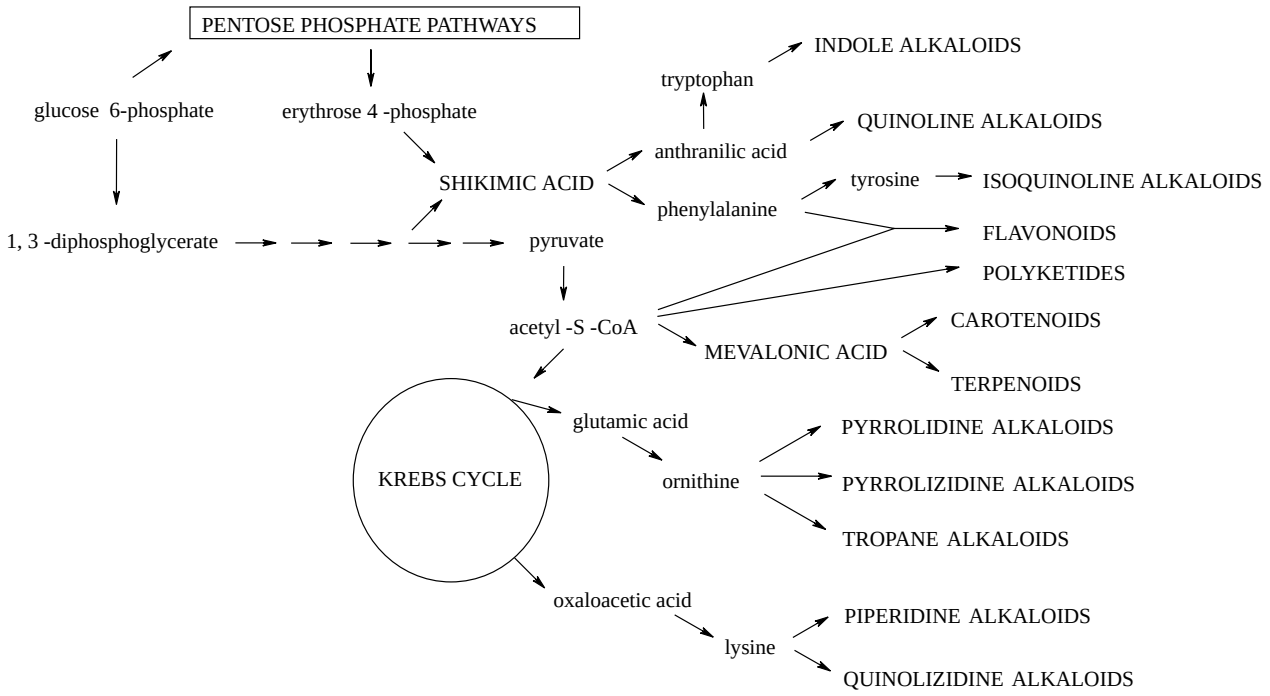

Fig. 1 The biosynthetic origin of secondary metabolites.

frequently offers a rational overview of the necessary chemical transformations. This may lead to isolation of the enzymes and perhaps the involvement of unanticipated intermediates. Although frequently these pathways are conserved, given the diversity of metabolic systems, it should not be assumed that the same compound will be produced by the same biosynthetic pathway in a different organism, i.e., in one plant family compared with another.

For biosynthetic experiments to be meaningful, the precursor must reach the site of synthesis at a time when the enzyme systems mediating metabolite formation are both present and active. Secondary metabolism occurs at a discontinuous rate in a given organism and at different points in the growth cycle in different organisms. Consequently, establishing a time for precursor administration to the organism is critical, if substantial degradation is to be avoided. Transportation and permeability factors may result in very low incorporations, even if the organism is known to be producing secondary metabolites at the time of the feeding and a known precursor is being used. Translocation of the precursor to the site of synthesis may be important in studies with whole plants or callus tissue. For microorganisms, metabolic degradation may occur because the growth of cell mass frequently precedes the initiation of secondary metabolite production.

Use of Radioisotopes

Although a single radiolabel may be adequate to demonstrate a preliminary precursor relationship through incorporation, it is preferable to use a precursor containing two, different, strategically placed labels. There are two types of these experiments, one in which two labeled precursors are physically mixed and the ratio of labels monitored. An example is a feeding experiment with [2-^{14}C, 4-$^{3}H_2$]-mevalonic acid. A second experiment involves using a precursor in which the two labels *are in the same molecule*; the use of [1,2-$^{13}C_2$]-acetate is an example. Double- or multiple-labeled substrates are used to examine bond-forming and bond-breaking reactions and to examine the stereospecificity of enzymatic processes if the precursor is labeled stereotopically (e.g., [4*R*-^{3}H]-mevalonic acid) and is selectively retained in the product or if the label in the product can be assigned stereotopically. Techniques are available for distinguishing between the prochiral hydrogens on a methylene or a methyl group.

Use and Detection of Stable Isotopes

The common stable isotopes used in biosynthetic studies are ^{13}C, ^{2}H, ^{15}N, and ^{18}O. Stable isotope-labeled

precursors have replaced radiolabeled precursors in many biosynthetic studies for the following reasons: 1) no appropriate radiolabeled isotope is available (e.g., N and O); 2) the detection methods frequently permit location of the label in the product directly; and 3) radiocontamination and safety issues are reduced. The negative aspects of stable isotope studies are: 1) detection methods are relatively insensitive (higher incorporation levels needed); 2) high levels of enrichment of the label in the precursor are required; and 3) reasonable quantities of the precursor are necessary, which can be expensive.

Mass spectrometry and NMR spectroscopy are the dominant techniques for detecting stable isotopes. MS offers the advantage that less sample is needed to establish incorporation, whereas NMR typically permits direct determination of the labeled site.

Administration of Precursors

When the system under examination is microbial, i.e., a plant in tissue culture or a cell-free system, administration of the precursor is straightforward. For meaningful conclusions, a profile of formation of the compound of interest over time is necessary so that feeding is conducted when there is active product formation. When intact plants are used, precursor feeding is more difficult; options are wick feeding through the stem, root feeding, isolated leaf feeding, or even direct injection into the stem.

Examining Intermediates

Conclusively establishing the role of potential intermediates in a biosynthetic pathway is a difficult aspect of biosynthesis. Typically, intermediates accumulate because subsequent enzymatic reactions are slow. Organisms also produce shunt metabolites that are off the main pathway and may not be further metabolized; these will also accumulate. Isolation of an "intermediate" does not, therefore, establish intermediacy. Trapping experiments are sometimes used to overcome these problems. In the pathway $A \Rightarrow B \Rightarrow C$, where A is a known precursor of C, labeled A and nonlabeled B are fed at the same time. The latter is metabolized to C and labeled B is produced from A; B is then temporarily available for isolation. An alternative approach for microbial metabolites is to mutate the organism or add specific enzyme inhibitors. This may allow intermediates to accumulate. Incorporation of a labeled, potential intermediate into a product does not prove that the intermediate lies on the main biosynthetic pathway. It may simply serve as a substrate for the enzymes involved. Only when each of the enzymes in a pathway has been isolated and characterized, and the substrate specificity determined, can the intermediates in a biosynthetic route be characterized.

Enzymes and Genes

Biosynthetic pathways are characteristically under enzymatic control and proceed with a very high degree of stereospecificity. Compared with the number of steps in the pathways of significant natural products, very few enzymes have been isolated and characterized and even fewer cloned and expressed. In the future, it will be very important to be able to express these enzymes heterologously in more productive systems so that these biocatalysts can be used for both known metabolite production and new metabolite generation.

One dream of the biosynthetic chemist is to develop a system of stabilized enzymes on solid supports, permitting a continuous flow process from precursors to products. With no variability due to climate or soil conditions, yields would be totally controllable and reproducible, and product clean-up would be greatly simplified, or ideally, unnecessary. With the isolation, characterization, cloning, and expression of more enzymes in biosynthetic pathways, the reality of the dream moves inexorably closer. Already the use of enzyme systems for directing stereospecific reactions in organic synthesis has risen dramatically, with a corresponding increase in efficiency and enantioselectivity.

Combinatorial Biosynthesis

Another biosynthetic dream is the ability to modulate predictably the product profile of an organism. In the microbial and plant tissue culture areas, this can be achieved randomly by modifying the growth medium or by challenging the organism with a chemical or other external agent (such as a fungus), producing metabolic stress metabolites (allelochemicals). A more controllable route to altering a metabolic profile in the polyketide area can be achieved through selectively modifying the gene sequence of the biosynthetic pathway. Known collectively as combinatorial biosynthesis, this way allows new products to be formed for chemical analysis and biological evaluation.

COMPOUNDS DERIVED FROM ACETATE

Acetyl coenzyme A is the biosynthetically active form of the two-carbon building block, acetate. It is of central

importance in mammalian, plant, and microbial biochemistry, giving rise to the fatty acids, the polyketides, and through mevalonic acid, the terpenes.

Fatty Acid Biosynthesis

The common fatty acids, such as palmitic (C_{16}), stearic (C_{18}), and arachidonic (C_{20}), have an even number of carbons. Their chain building process initially involves a reaction of acetyl CoA with carbon dioxide to afford the more chemically reactive malonyl CoA, which condenses with a second acetate unit. The carbon dioxide subsequently lost is the same carbon that was added. It is this specificity that allows correlative experiments with [1,2-$^{13}C_2$]-acetate. Reduction of the beta carbonyl group is followed by dehydration and reduction of the *cis*-olefin to the saturated fatty acid. Repetition causes chain extension by two-carbon fragments (Fig. 2). Unsaturated fatty acids can also result from dehydrogenation, as shown in the conversion of oleic acid to arachidonic acid. The latter compound is the precursor of the prostaglandins (Fig. 3). Branching in fatty acids may occur either through the initiating acid, through acylation with a preformed fatty acid, or through reaction of an intermediate olefin with methionine.

The Polyketide Pathway

Aromatic compounds are predominantly formed through either the shikimate (vide infra) or the polyketide pathway. Collie first suggested the polyketide pathway in 1893, and this was extended theoretically (acetate hypothesis) and experimentally by Birch. Factors involved in the diversity of products include the chain-initiating unit, the number of units in the cyclizing chain, condensation reactions occurring between separately formed polyketide chains,

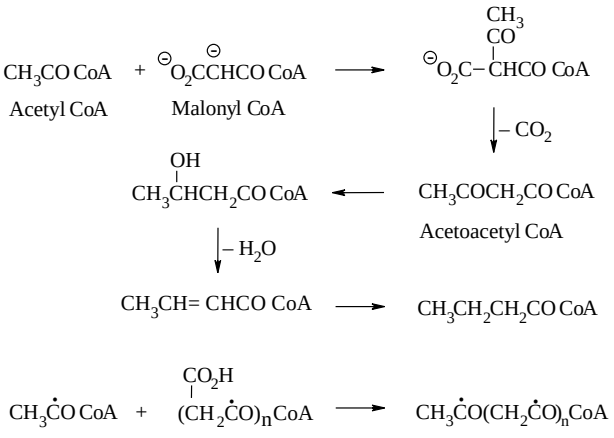

Fig. 2 The biosynthesis of fatty acids.

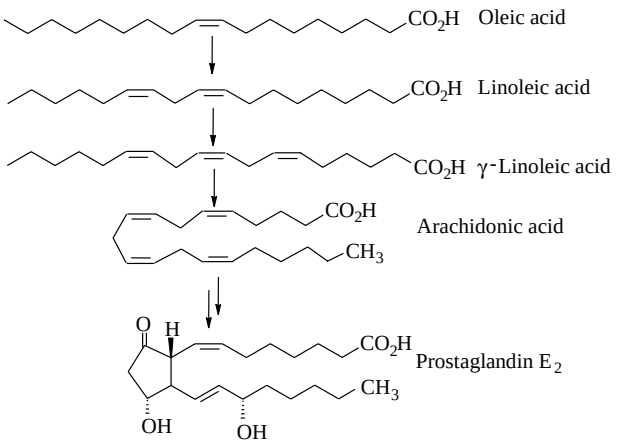

Fig. 3 The biosynthesis of prostaglandins.

and secondary processes, such as alkylation or halogenation. The 1,3-diketone nature of the intermediate chain leads to a characteristic *meta*-relationship between ether or phenolic groups; in shikimate-derived metabolites these groups are typically *ortho*-related.

Penicillic acid provides an example of a simple tetraketide whose aromatic ring is cleaved and cyclized, as shown by experiments with [1,2-$^{13}C_2$]-acetate (Fig. 4). On the other hand, the pentaketide citrinin is the result of the cyclization of a linear polyketide chain wherein three methyl groups are introduced from methionine (Fig. 5). Hexaketide derivatives are rare. The naphthoquinone plumbagin is an example where cyclization followed by decarboxylation occurs. Griseofulvin is a heptaketide (Fig. 6) and was one of Birch's very early demonstrations of the accuracy of the acetate hypothesis. Anthraquinones, such as islandicin, are octaketide derivatives; the two alternative modes of cyclization of the polyketide chain

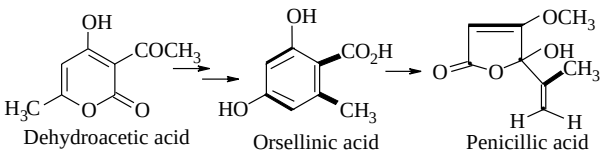

Fig. 4 The biosynthesis of penicillic acid.

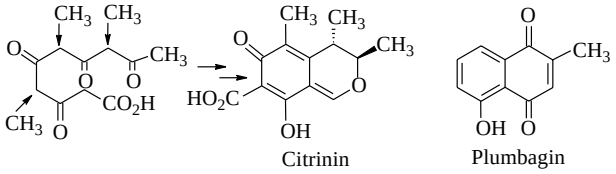

Fig. 5 The biosynthesis of citrinin.

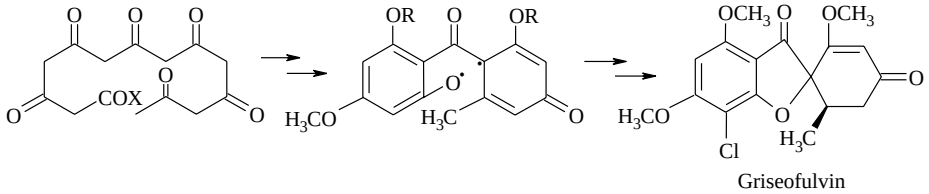

Fig. 6 The biosynthesis of griseofulvin.

can be distinguished through the use of [1,2-$^{13}C_2$]-acetate. Xanthones can be produced through oxidative cleavage of the quinone ring, cyclization and decarboxylation (Fig. 7).

The most clinically significant polyketides are the anthracyclinone and tetracyclinone antibiotics produced in *Streptomyces* cultures. The tetracyclines demonstrate that chain initiation can occur with a malonamide unit and that a wide range of reactions can occur after the initial aromatic cyclization (Fig. 8). Mixed biosynthesis is very evident in the macrolide antibiotics, where various combinations of acetate and propionate form the chain (e.g., tylosin and nystatin) or only propionate (e.g., erythromycin) and cyclize to a ring of varying size. Several plant-derived anthraquinones are of clinical significance, including the sennosides of senna (*Cassia angustifolia*), the aloins of aloe (*Aloe vera* and related species), the cascarosides of cascara sagrada (*Rhamnus purshiana*), and hypericin of Saint John's Wort (*Hypericum perforatum*; Fig. 9).

SHIKIMATE PATHWAY

The majority of aromatic compounds, including most alkaloids, are derived through the shikimate pathway. At the branching point of chorismic acid, either anthranilic acid, the precursor of tryptophan, or prephenic acid, the precursor of phenylalanine, itself the precursor of tyrosine and dopa (3,4-dihydroxy-phenylalanine), is formed (Fig. 10). Phosphorylation at the 3-position, condensation with phosphoenolpyruvate, and elimination of phosphoric acid yields chorismate from shikimate. Chorismate is also the precursor of a number of simple, and very important, aromatic compounds, including salicylic acid, 4-amino-benzoic acid (PABA), a constituent of folic acid, and 2,3-dihydroxybenzoic acid, a key acylating group of enterobactin.

Amination at the 2-position and loss of pyruvate yields anthranilic acid, the precursor of the quinoline alkaloids, distributed widely in the Rutaceae, and tryptophan, the precursor of the indole alkaloids (Fig. 10). Internal Claisen rearrangement on chorismic acid yields prephenic acid en route to phenylalanine. During the course of the hydroxylation of phenylalanine to tyrosine, there is a characteristic NIH shift of the proton at C-4′ (Fig. 11). Further hydroxylation yields dopa, used in the treatment of Parkinson's disease. Dopa can oxidize and polymerize to yield the melanin group of hair, skin, and eye pigments. 4′-amination of chorsimic acid, a Claisen rearrangement, and amination yields 4′-aminophenylalanine, whose importance is as a precursor of chloramphenicol (Fig. 12).

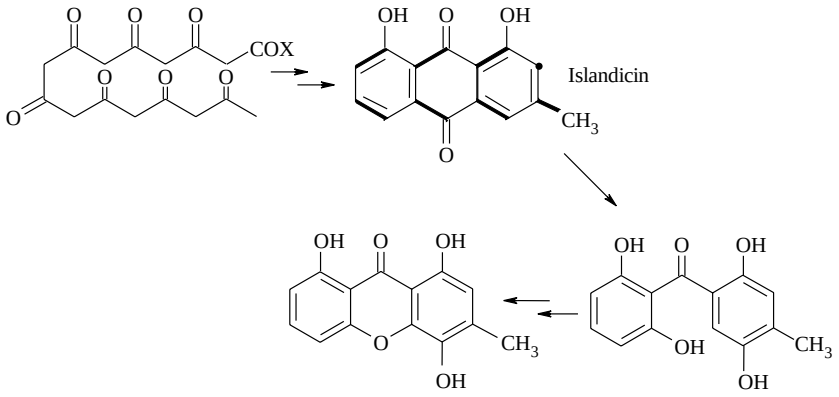

Fig. 7 The biosynthesis of anthraquinones and xanthones.

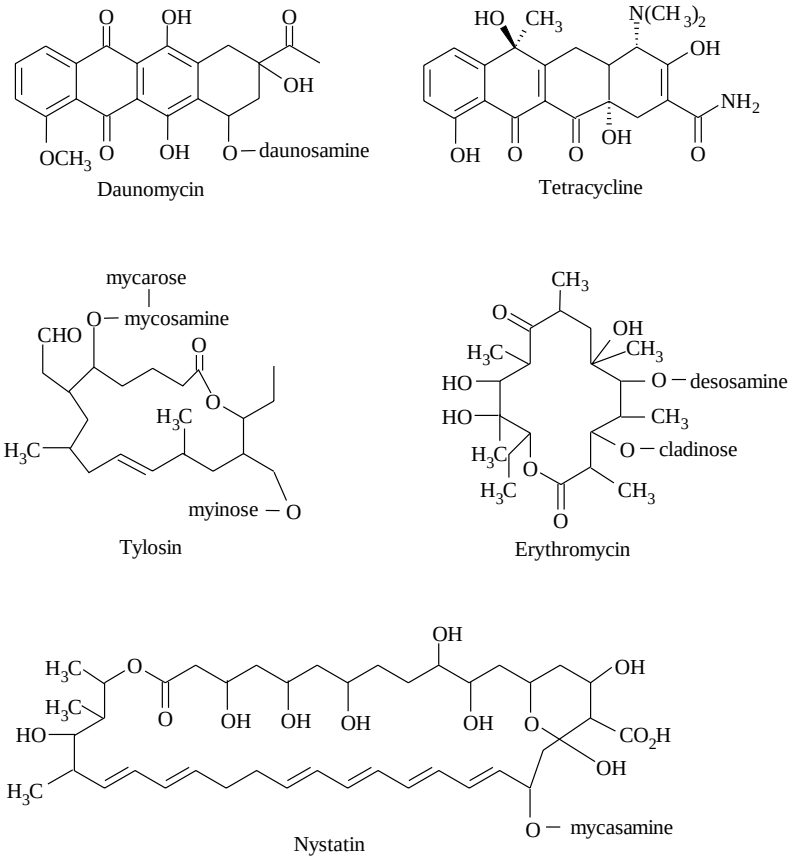

Fig. 8 Representative polyketide antibiotics.

Oxidative deamination of phenylalanine by phenylalanine ammonia lyase (PAL) and 4-hydroxylation affords *p*-coumaric acid, whose derivatives are the fundamental building blocks of lignin, as well as the lignans, such as the potent anticancer agent podophyllotoxin (Fig. 13). The latter is the template for the drugs, teniposide and etoposide.

2′-Hydroxylation of *p*-coumaric acid, followed by photocatalyzed isomerization of the double bond and

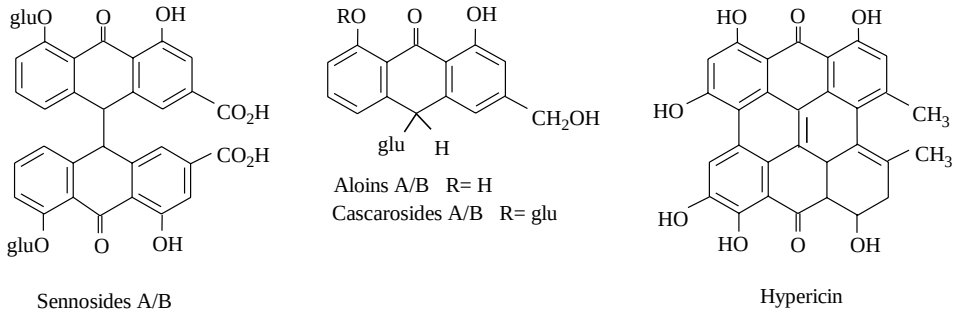

Fig. 9 Representative plant-derived anthraquinones.

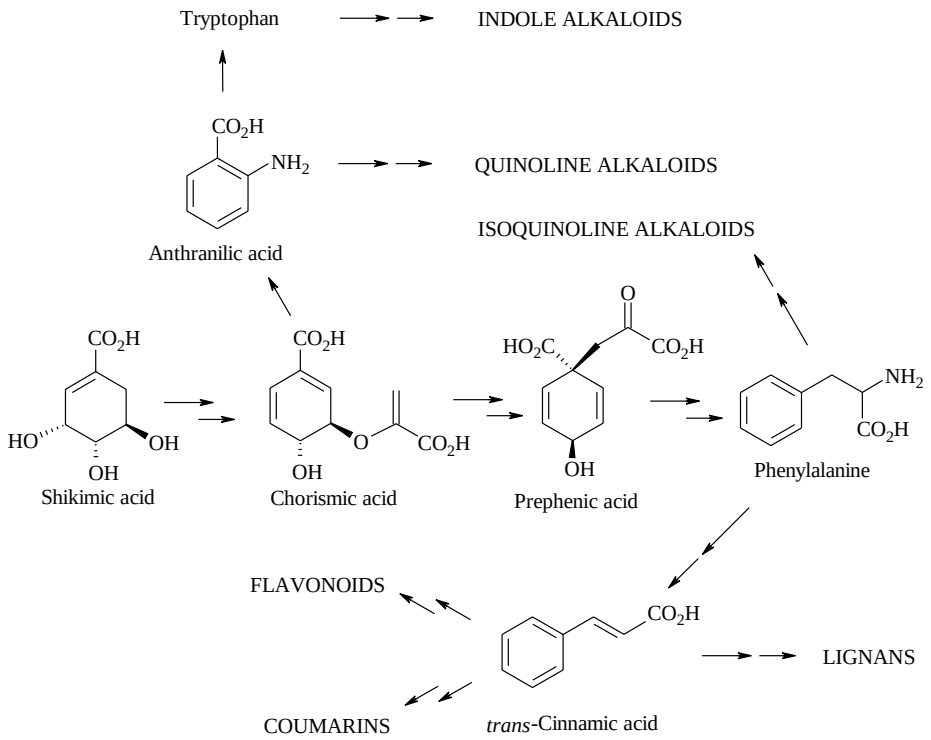

Fig. 10 The relationships of shikimate-derived compounds.

lactonization, affords 7-hydroxy coumarin (umbelliferone). Prenylation at the 6-position, epoxidation, cyclization, and a retro-aldol reaction afford the furanocoumarins (Fig. 14); some (psoralen, bergapten) are known as photosensitizers and find use in the treatment of vitiligo and psoriasis. Coumarin stimulates the reticulo-endothelial system and served as a model for anticoagulant drugs, such as dicoumarol and warfarin.

Flavonoids are essentially universal plant pigments and exist in at least nine different structure classes, frequently with attached sugar units. They are derived from a mixed acetate-shikimate biosynthesis. 4-Coumaroyl CoA reacts with a triketide unit to afford 4,2′,4′,6′-tetrahydroxychalcone, which cyclizes to naringenin under the influence of chalcone isomerase. 3-Hydroxylation and dehydrogenation leads to the

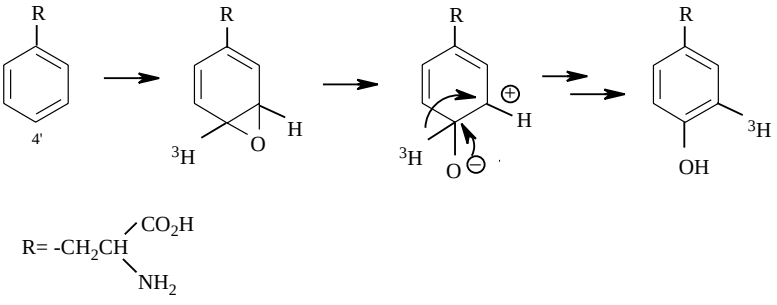

Fig. 11 The NIH shift in the 4′-hydroxylation of phenylalanine.

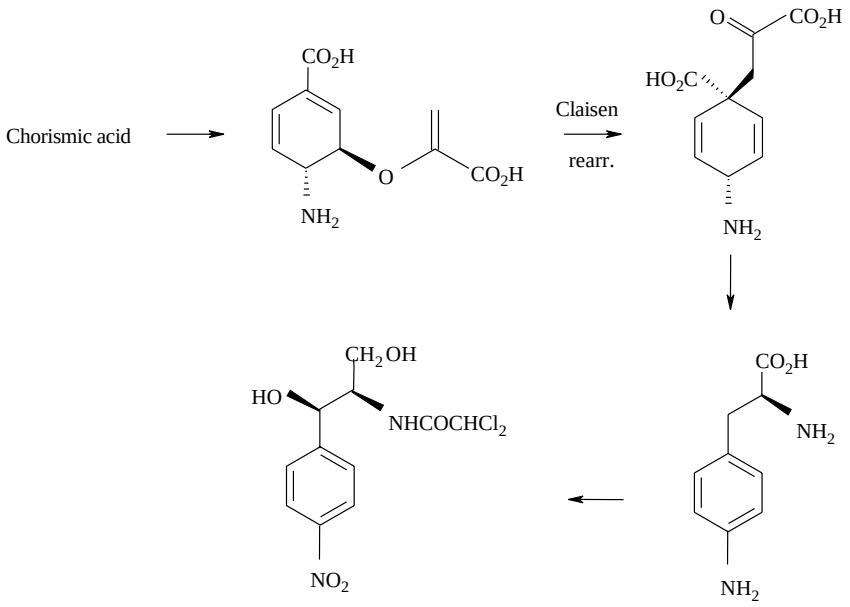

Fig. 12 Shikimate-derived metabolites.

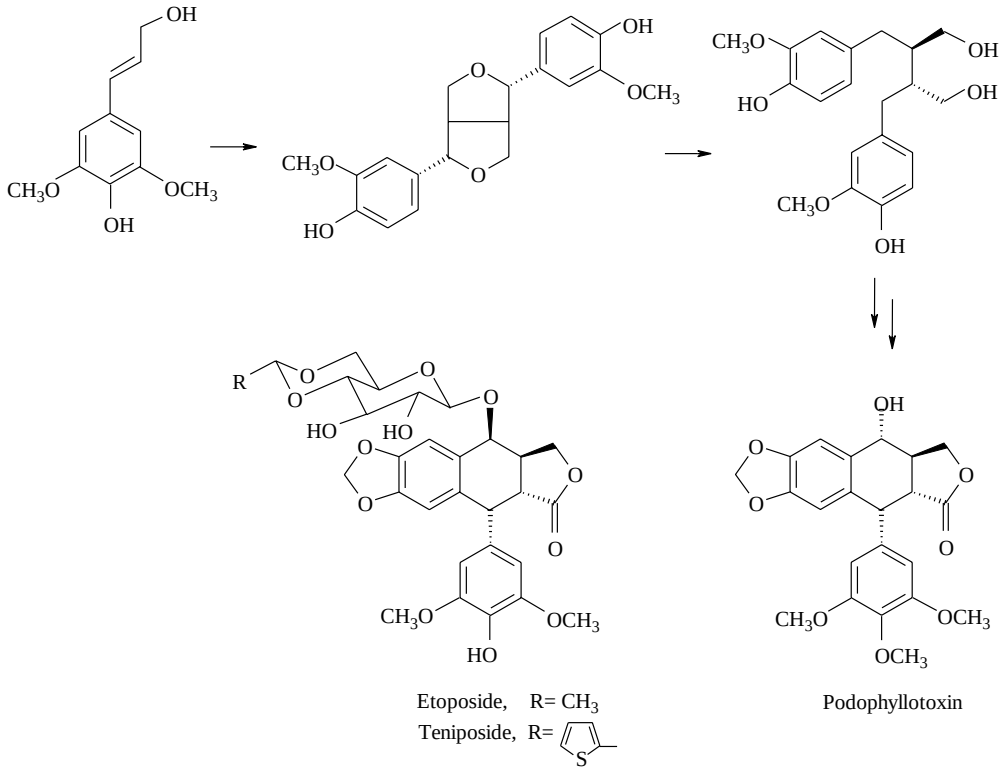

Fig. 13 The biosynthesis of lignans.

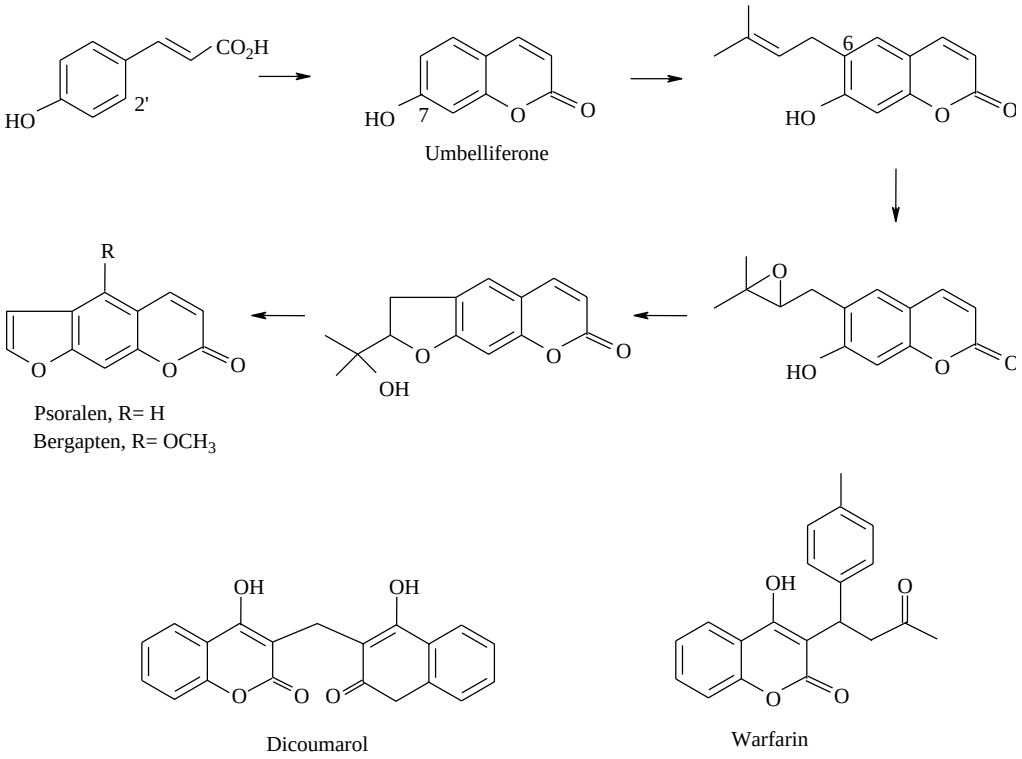

Fig. 14 The biosynthesis of furanocoumarins.

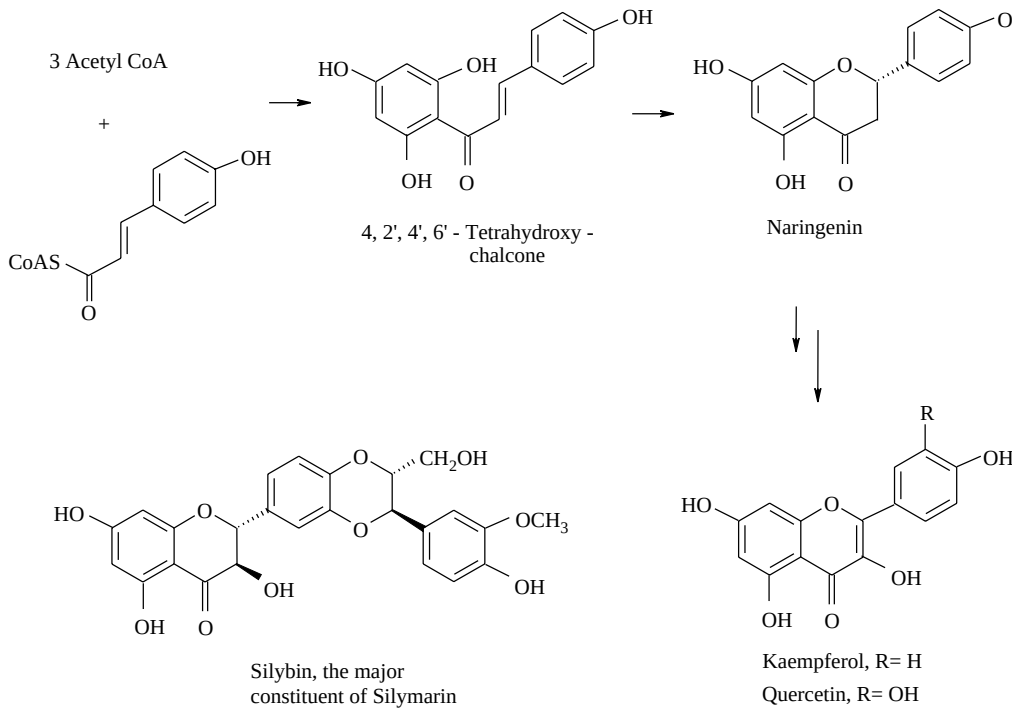

Fig. 15 The biosynthesis of flavonoids.

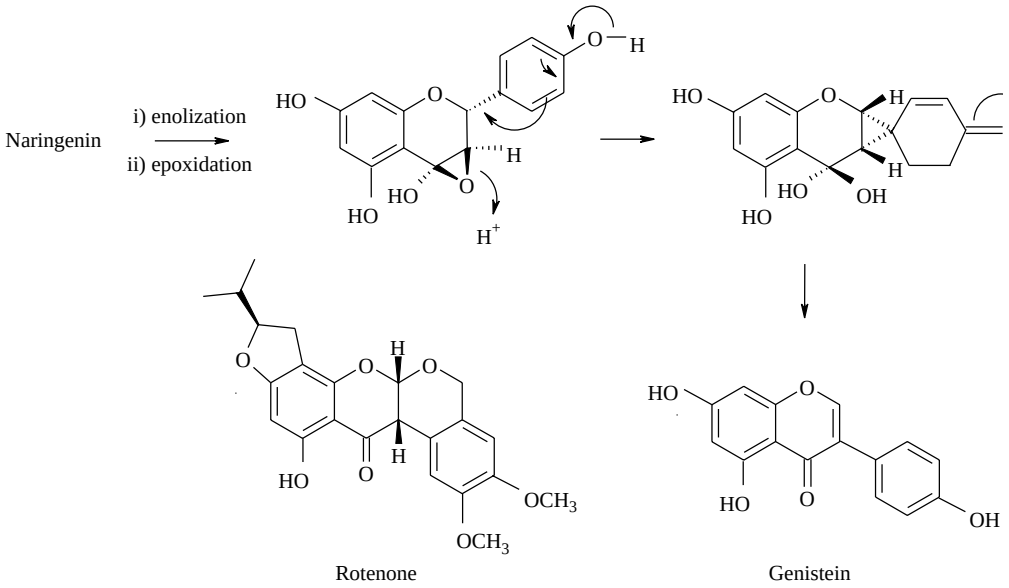

Fig. 16 The biosynthesis of isoflavonoids.

flavanol, kaempferol (Fig. 15), which, along with quercetin (3′-hydroxykaempferol), is widely distributed. There is very substantial interest in the flavonoids present in the diet for their wide range of in vitro activities. *Silybum marianum* (milk thistle) is used in Europe as an antihepatotoxic agent for mushroom poisoning, where the active ingredient is silymarin (a mixture of flavonolignans).

Isoflavonoids are of limited distribution (Fabaceae, bean family) and are probably formed through the epoxidation and rearrangement of the enol form of a flavone (e.g., the conversion of naringenin to genistein; Fig. 16). Several isoflavonoids are associated with strong estrogenic activity, and there is interest in their potential in the prevention of hormone-dependent breast cancer. Rotenoids (e.g., rotenone) from *Derris* and *Tephrosia* species are noted for their insecticidal and cytotoxic activity.

COMPOUNDS DERIVED FROM ISOPENTENYL PYROPHOSPHATE

Numerous natural products contain units derived from a terpene precursor. Built up of five carbon "isoprene" units, they are successively known as hemiterpenes (C_5), monoterpenes (C_{10}), sesquiterpenes (C_{15}), diterpenes (C_{20}), sesterterpenes (C_{25}), triterpenes (C_{30}), and tetraterpenes (C_{40}). Successive units may join through head-to-tail (e.g., farnesol) or tail-to-tail linkages (e.g., squalene). Isopentenyl pyrophosphate (IPP), derived from either mevalonic acid or 1-Deoxyxylulose, is the moiety isomerizing to dimethylallyl pyrophosphate (DMAPP) and is also the chain extending unit. Fig. 17 shows the relationships between these terpenes and how the steroids are derived from a triterpene precursor. The formation and occurrence of these metabolites is widespread, and many derivatives are essential for mammalian functions (e.g., cholesterol, steroid hormones, bile acids, vitamin D, and retinols).

Geraniol is the primordial monoterpene, and its simple derivatives and cyclization products occur in the oils of many plants, used as flavoring and aromatic agents (e.g., caraway, coriander, dill, eucalyptus, lavender, orange, peppermint, rose, and sandalwood), as well as drugs (e.g., camphor, menthol), and insecticides (e.g., pyrethrins). Chain extension leads to farnesol, which can dimerize to squalene or undergo its own molecular modifications to yield the diverse sesquiterpenes. One of these, artemisinin from *Artemisia annua*, is of significance as an antimalarial agent, and another, (−)-gossypol, is a male contraceptive agent (Fig. 18).

Several diterpene derivatives are commercially significant drugs. Forskolin, from the Indian medicinal plant

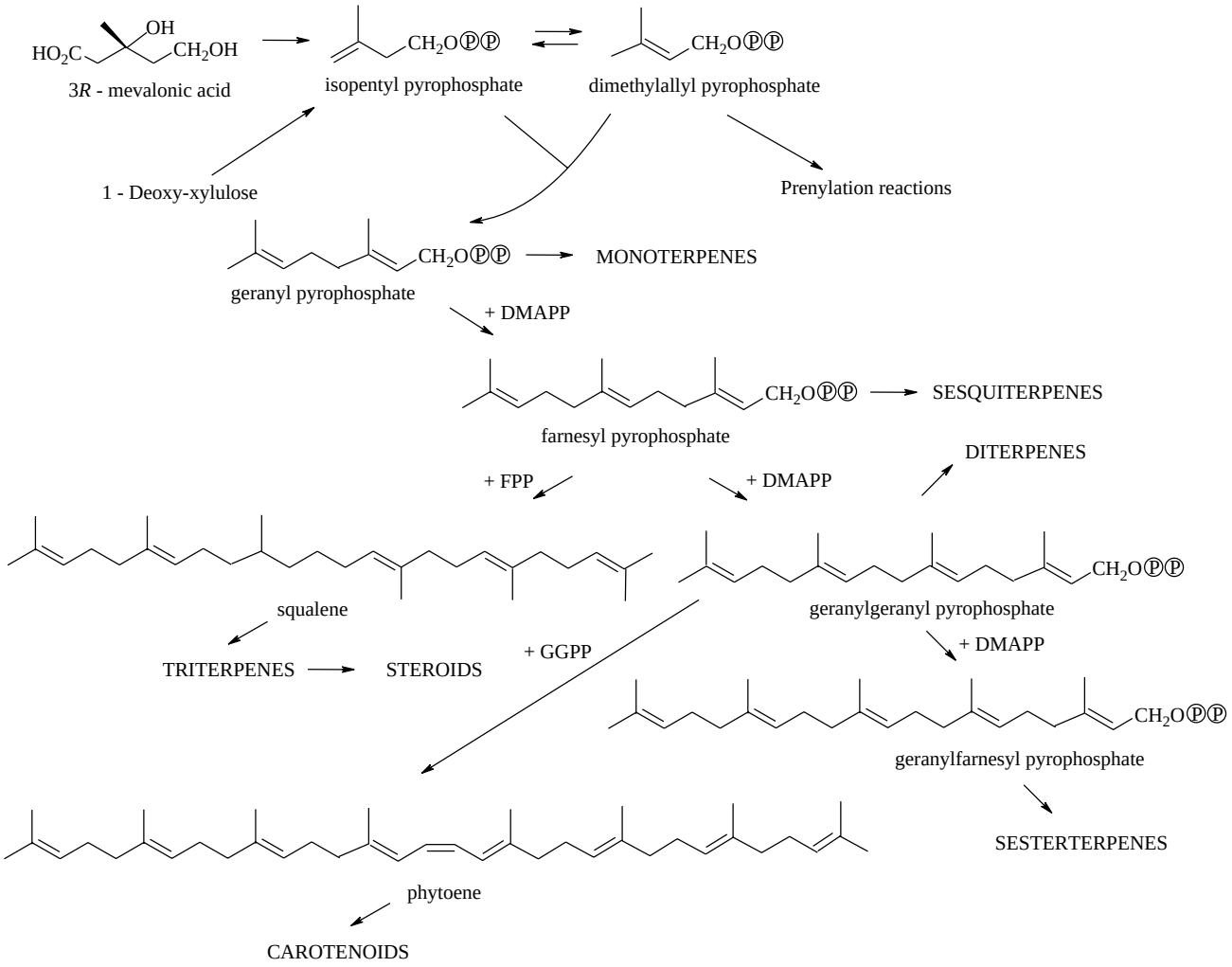

Fig. 17 The relationships of the isoprene-derived compounds.

Coleus forskohlii, is a potent inhibitor of adenylate cyclase and shows promise for congestive heart failure and bronchial asthma. The gingkolides, from the leaves of *Gingko biloba*, are potent inhibitors of platelet activating factor, and in a specified mixture with associated flavonoids, they improve peripheral and cerebrovascular function, hence, their wide use for senile dementia and memory loss. Taxol, originally isolated from the yew *Taxus brevifolia*, is a potent agent against many forms of cancer, including ovarian and breast cancer. It acts by promoting the assembly of microtubules (compare podophyllotoxin, colchicine, and vincristine). Geranylgeranyl units are also found in the side chains of chlorophyll a and vitamin K_1. Many *Euphorbia* species have a latex containing tigliane esters noted for their powerful skin irritant and cocarcinogenic activity. Derivatives of an *ent*-kaurene alcohol (stevioside, rebaudioside) are non-cariogenic sweetening agents.

Through a series of cyclizations, squalene oxide (C_{30}) affords lanosterol in animals and fungi and cycloartenol in plants (Fig. 19). In both instances, the intermediate is a protosteryl cation that can also undergo a series of Wagner-Meerwein rearrangements to afford the cytotoxic cucurbitacins of melons and cucumbers. Squalene oxide in a chair-chair-chair-boat conformation yields the dammarenyl cation, a parent of numerous triterpene skeleta (e.g., lupane, oleanane, ursane, and taraxerane) contained in the saponins found in many foodstuffs, in soaps, and in several drugs from complementary systems of medicine (e.g., ginseng, liquorice, Bupleurum, and horsechestnut).

Steroids are degraded triterpene derivatives, and the different nuclei are classified based on carbon number.

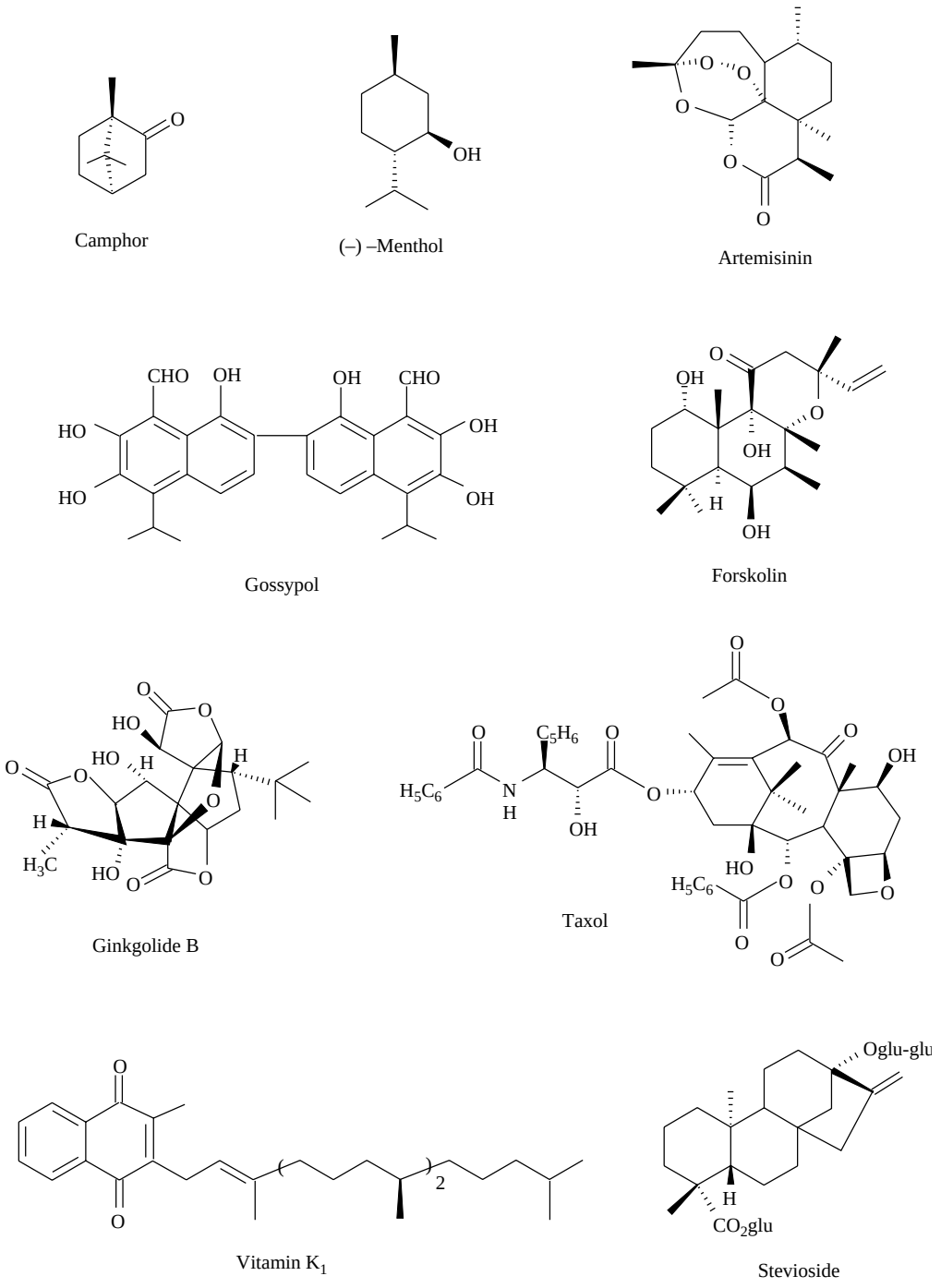

Fig. 18 Representative mono-, sesqui- and diterpene derivatives.

The most significant, from a drug perspective, are the cholane, pregnane, androstane, and estrane systems. When a carbon, such as a methyl group, is lost from the nucleus, the term *nor* is used.

In animals, lanosterol undergoes a series of degradative steps (Fig. 20), whose sequence depends on the organism to afford cholesterol. In photosynthetic organisms, this role is played by cycloartenol where the cyclopropane ring

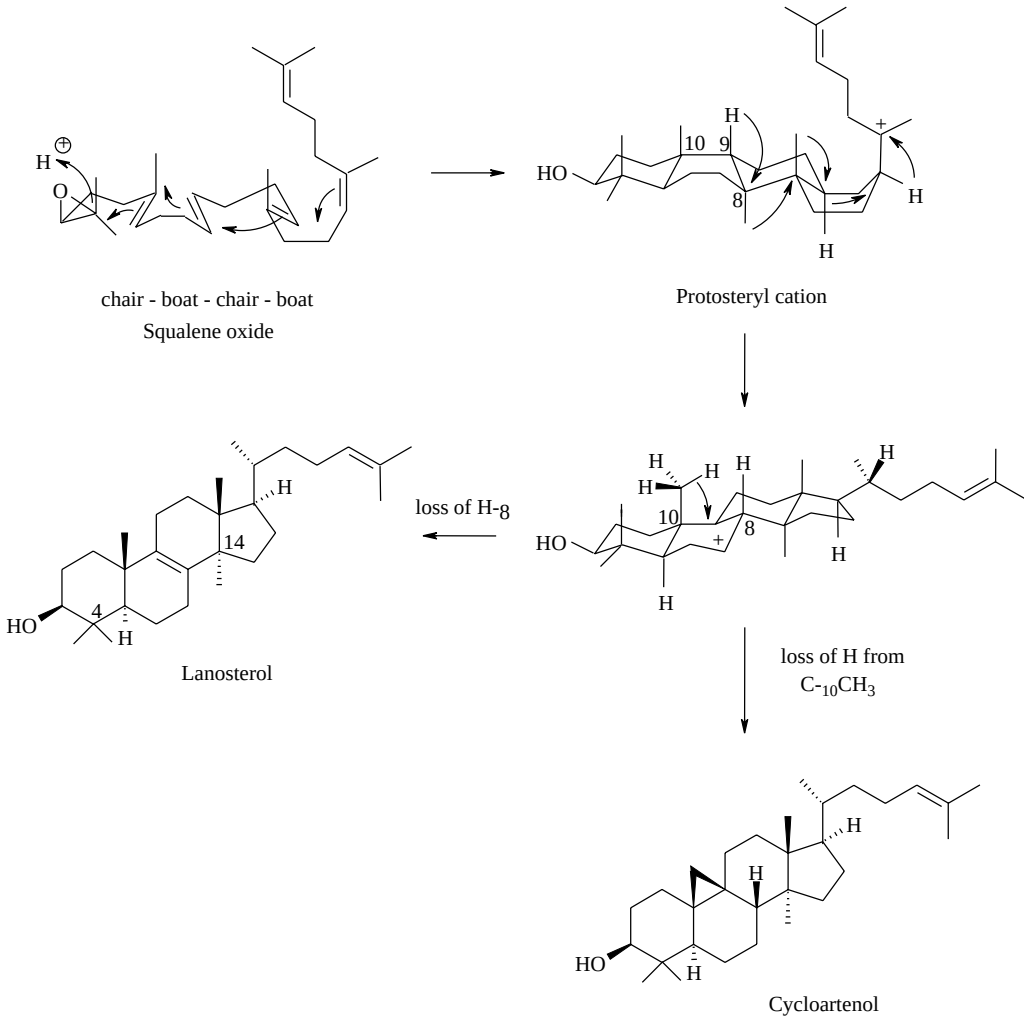

Fig. 19 The biosynthesis of lanosterol and cycloartenol.

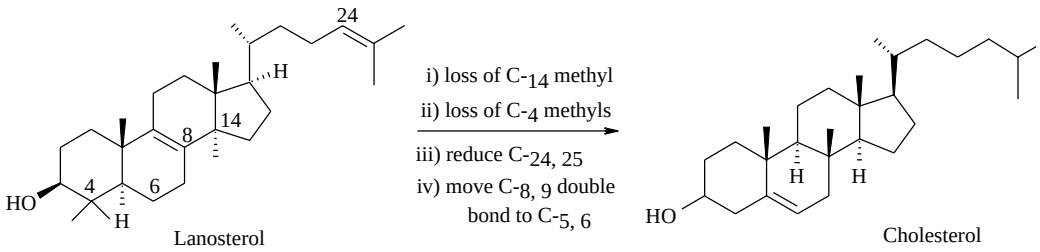

Fig. 20 Steps in the pathway from lanosterol to cholesterol in mammals.

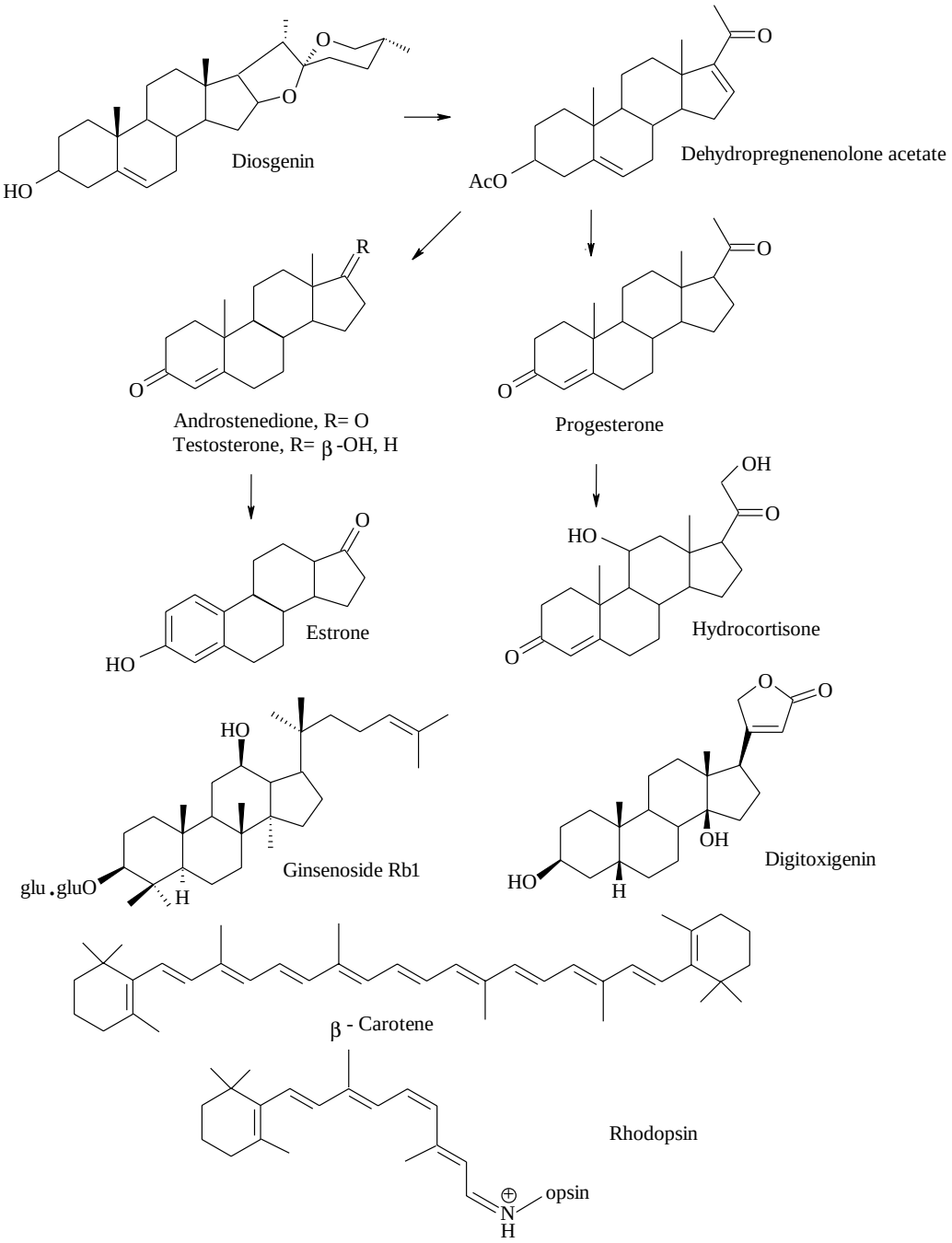

Fig. 21 Representative degraded triterpenes and steroids, and the carotenoids.

is opened. Cholesterol is in almost every animal tissue, and is derived from cattle brains and spinal chords, as well as lanolin from sheep wool. High levels of blood cholesterol are correlated with a high risk of heart disease and atherosclerosis (through deposition of cholesterol and its esters in the artery wall). Thus, an agent that can selectively inhibit an early stage (HMG-CoA reductase) in cholesterol biosynthesis in humans, such as mevinolin (lovastatin), has the effect of reducing serum cholesterol levels.

The steroidal saponins, typically based on a C_{27} sterol nucleus, are distributed in the Dioscoreaceae, the Agavaceae, and the Liliaceae, and have a spiroketal at C-22 (e.g., diosgenin). They are also characterized by numerous sugar units attached at C-3 and sometimes elsewhere. Although not employed as drugs, steroidal saponins are critical for the semisynthesis of important hormones (estrogens, androgens, and progestins) and selected anti-inflammatory agents. An example is the conversion of diosgenin to a dehydropregnenolone acetate for elaboration to the hormones (progesterone, testosterone, androstenedione, and estrone) and the corticosteroids. Microbial transformations are important in several of the reaction sequences. Ginsenosides, the adaptogenic principles of Korean ginseng (*Panax ginseng*), are polysaccharide derivatives of a trihydroxylated (3β, 12β, and 20*S*) dammarane nucleus, and sugar variation occurs at C-3 and C-20 (Fig. 21).

Cardiac glycosides, such as those of *Digitalis lanata*, are composed of a polysaccharide unit of three or four sugars, including some 2,6-dideoxyhexoses, linked at C-3 to a modified polyhydroxy (C-3β, C-12β, and C-14β in the case of digitoxigenin) steroid nucleus. The modification takes place on a 20-keto-pregnane through hydroxylation, the addition of acetate, and cyclization to yield an α,β-Unsaturated butyrolactone. Cucurbitacins, withanolides, ecdysones, guggulsterone, limonoids, and quassinoids are also modified triterpene derivatives with potent biological effects, including high cytotoxicity (Fig. 21).

The carotenoids are tetraterpenes and are formed through the tail-to-tail coupling of geranyl pyrophosphate, followed by cyclizations at each terminal (e.g., β-Carotene in carrots). They are widely used as coloring agents for foods, confectionery, and drugs. β-Carotene is under investigation as an antioxidant for the prevention of cancer. Cleavage of β-carotene yields retinol (vitamin A_1; Fig. 21). The retinoids are important signalling agents and regulate many aspects of cell differentiation, embryonic development, growth, and vision (rhodopsin is a derivative of 11-*cis*-retinal with the protein opsin).

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ALKALOIDS

Alkaloids are nitrogenous secondary metabolites primarily derived from amino acids for both their nitrogen content and a portion of their carbon framework. However, the approximately 27,000 known alkaloids defy a simple definition. Many alkaloids and their derivatives display

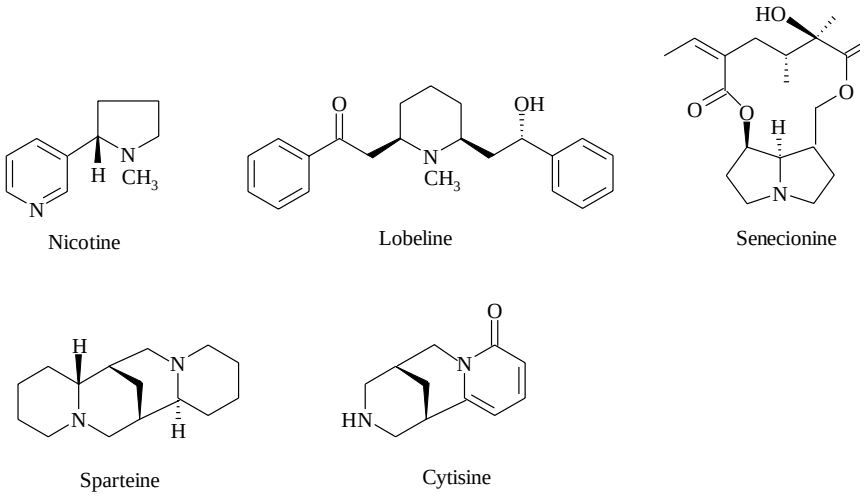


Fig. 22 Representative ornithine- and lysine-derived alkaloids.

profound biological effects and are of enormous commercial, pharmaceutical, and social significance. Ornithine, lysine, phenylalanine, and tryptophan are the principal amino acid precursors.

ALKALOIDS DERIVED FROM ORNITHINE AND LYSINE

There are three principal groups of alkaloids derived from ornithine: nicotine, the pyrrolizidines, and the tropanes, all having significant biological effects. From lysine are derived the piperidine alkaloids (e.g., lobeline, an antismoking agent) and the quinolizidine alkaloids, such as sparteine (an oxytocic agent) and cytisine (a teratogenic agent; Fig. 22). Only the alkaloids derived from ornithine will be discussed here.

Two very different taxa, the Asterales (Solanaceae) and Geraniales (Erythroxylaceae), formulate the tropane nucleus through similar, but distinct, pathways. Using[2-^{14}C]-ornithine in solanaceous plants, the bridgehead carbon C-1 was labeled, precluding an unbound symmetrical intermediate. In *Atropa belladonna*, ornithine is *N*-methylated prior to decarboxylation to *N*-methylputrescine. By contrast, in *Erythroxylum coca*, the bridgehead carbons C-1 and C-5 were equally labeled, suggesting that an unbound putrescine is methylated. Oxidative deamination affords an aldehyde that undergoes Mannich closure to yield *N*-methyl-pyrrolinium; condensation with acetoacetate affords hygrine-1′-carboxylic acid.

Decarboxylation is followed by oxidative cyclization to tropinone, followed by stereospecific reduction to the α-Hydroxy group, which is esterified to hyoscyamine. The esterifying ester, tropic acid, is an intramolecularly rearranged phenyllactic acid (derived from phenylalanine). Further elaboration of hyoscyamine yields scopolamine (Fig. 23). The enzymes for this transformation are known.

In the biosynthesis of cocaine, the carboxylic acid is retained as the methyl ester, and after stereospecific reduction to afford the β-alcohol, benzoylation affords cocaine (Fig. 23). Although cocaine is widely recognized as a drug of abuse, for many populations in South America, the chewing of coca leaves is a routine aspect of the working day and has been for thousands of years.

There are no drugs based on the pyrrolizidine alkaloids of the Asteraceae (e.g., *Senecio* and *Symphytum*) and Boraginaceae (*Crotolaria*). However, these alkaloids pose a great threat to human and animal health because of their potential for inadvertent consumption. In the case of 1,2-dehydro derivatives, such as senecionine, ingestion leads to nonreversible hepatotoxicity. Pyrrolizidine nucleus formation from two units of ornithine is shown (Fig. 24).

ALKALOIDS DERIVED FROM PHENYLALANINE AND TYROSINE

The isoquinoline alkaloids are the second largest group of alkaloids, numbering about 6000, and can be viewed as five

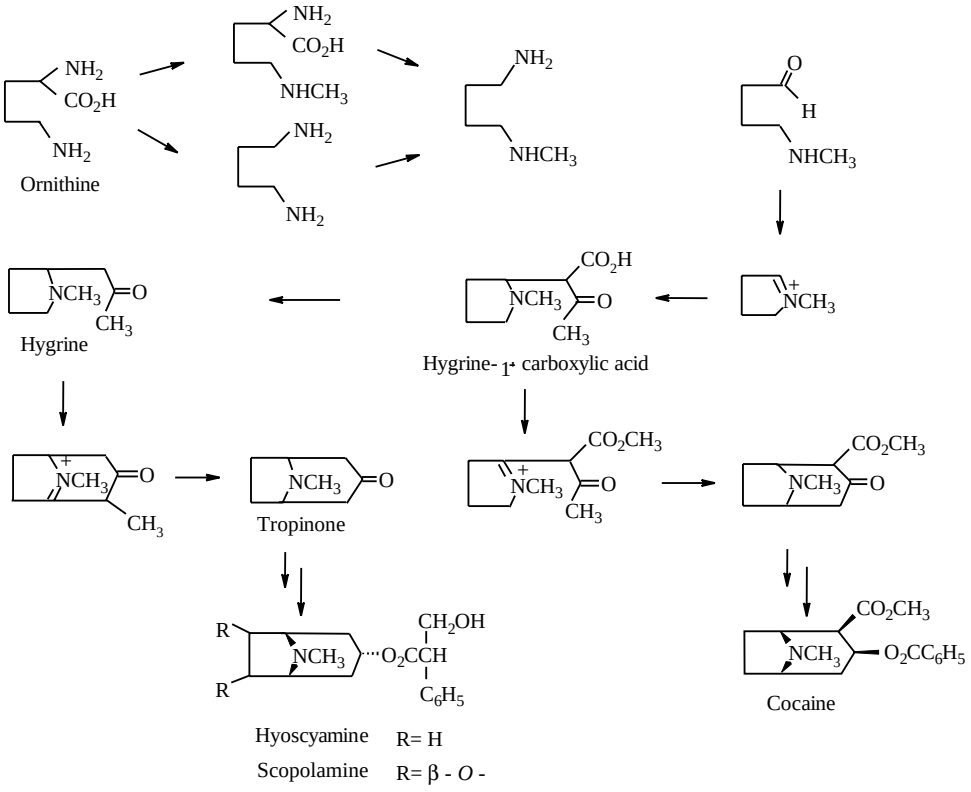


Fig. 23 The biosynthesis of the tropane alkaloids.

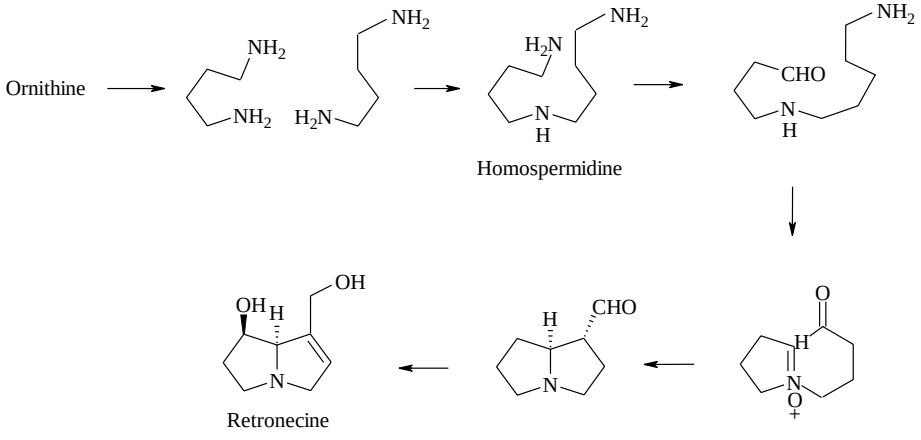


Fig. 24 The biosynthesis of the pyrrolizidine alkaloids.

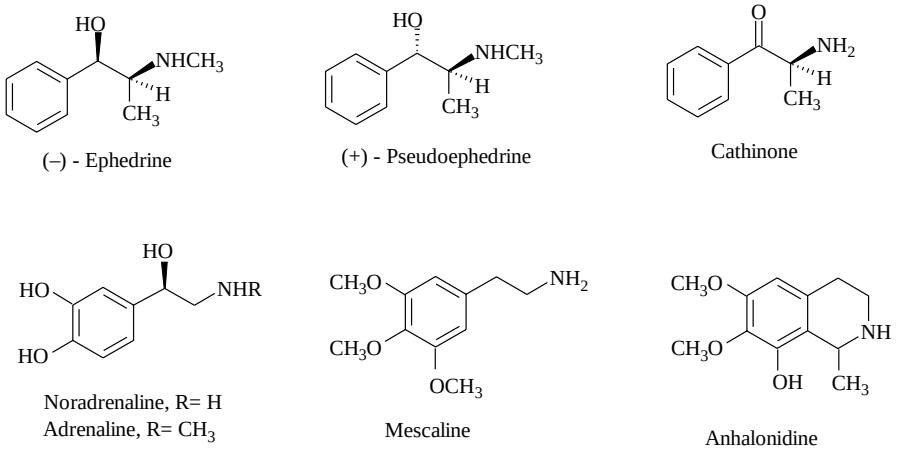


Fig. 25 Simple alkaloids derived from phenylalanine and tyrosine.

subgroups—the simple tetrahydroisoquinolines, the benzylisoquinolines, the phenethylisoquinolines, the Amaryllidaceae alkaloids, and the monoterpene isoquinolines. In addition, there are a number of simple phenethylamine derivatives, including ephedrine (originally from *Ephedra* species, but now synthesized) and pseudoephedrine, used for asthma and nasal decongestion, respectively. Khat (*Catha edulis*) is widely used as a stimulant in the southeastern Arabian peninsula and contains cathinone. Tyrosine is the precursor of the neurotransmitter noradrenaline and the hormone adrenaline. The hallucinogen mescaline, from the mushroom *Lophophora williamsii*, is also a member of this series (Fig. 25).

An aldehyde, 4-hydroxy-phenylacetaldehyde, operating under the influence of the enzyme norcoclaurine synthase, condenses with dopamine to afford (*S*)-norcoclaurine, the progenitor of all benzylisoquinoline alkaloids (Fig. 26). Robinson, in 1917, was the first to suggest the biogenetic derivation of the pavines, aporphines, morphinans, and protoberberines from a benzylisoquinoline precursor. These ideas led to a correct proposal for the structure of morphine and were expanded to embrace numerous alkaloid classes (Fig. 27).

Papaverine is one of the few commercial alkaloids synthesized, rather than isolated. It is used either alone for various vascular disorders or in combination as an antispasmodic. The several classes of bisbenzylisoquinoline alkaloid are based on the number of bridges between the units and their orientation. The alkaloids are common in the Menispermaceae and the Ranunculaceae. One member of the series, tubocurarine, is the prototype for several neuromuscular blocking agents; an activity based

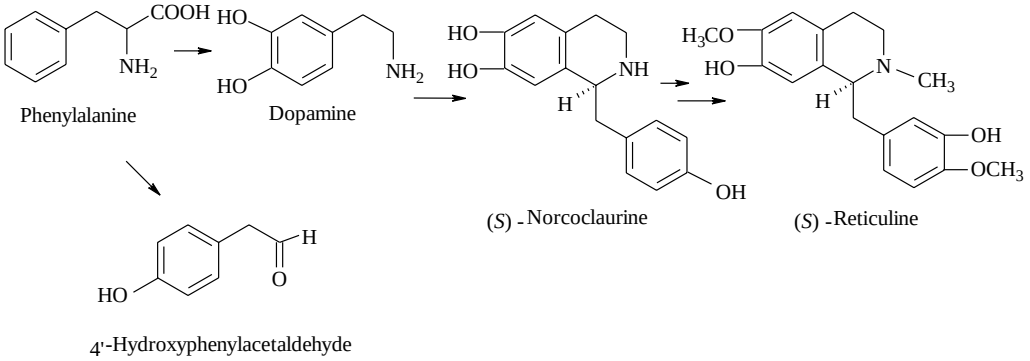


Fig. 26 The biosynthesis of (*S*)-norcoclaurine and (*S*)-reticuline.

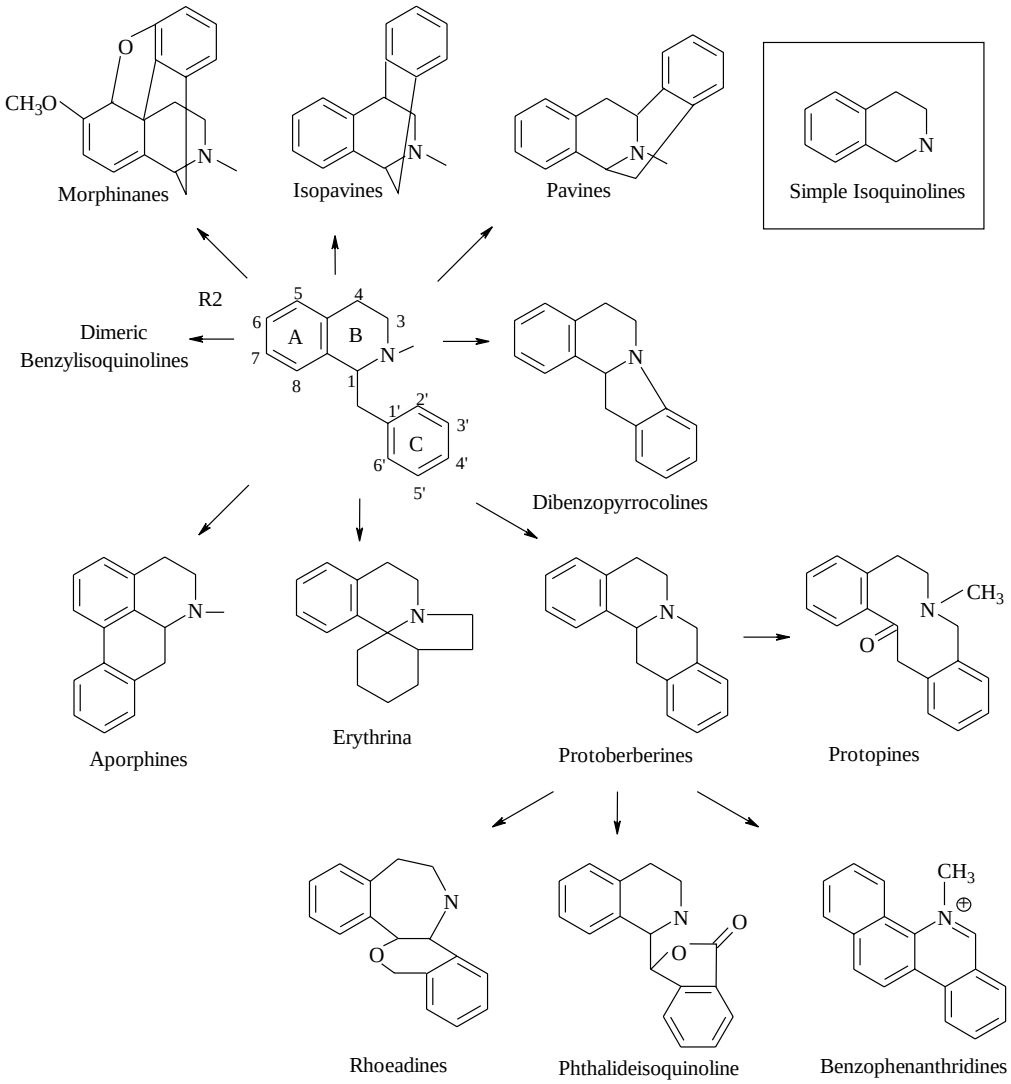


Fig. 27 Biosynthetic interrelationships of the major classes of benzylisoquinoline alkaloids.

on the ethnobotanical use of the tube curares (*Chondrodendron* species) is its use as arrow poisons in the upper Amazon.

Phenol oxidative coupling was proposed by Barton and Cohen in 1957 to enumerate the relationships between many of the benzylisoquinoline alkaloid groups (Fig. 27) and accounts for the importance of reticuline as a precursor. In the case of berberine, phenolic oxidative coupling occurs between the *ortho* benzylic position and the *N*-methyl carbon (berberine bridge) to afford scoulerine (Fig. 28). Many of the enzymes in this pathway have been elucidated by Zenk and coworkers. The protoberberines themselves are the precursors of several groups of alkaloids, including the protopines, the benzophenanthridines (e.g., sanguinarine, used as an antiplaque agent) and the phthalideisoquinolines (e.g., β-Hydrastine, a constituent of *Hydrastis canadensis*; Fig. 29). Berberine is a widely used antimicrobial agent, being active against *Staphylococcus, Streptococcus, Proteus, Vibrio*, etc. (Fig. 28).

Morphine and related alkaloids are specific to the genus *Papaver* (Berberidaceae), although the antipodal series of alkaloids is distributed in the Menispermaceae. Early in the biosynthesis of morphine, an inversion at C-1 of (*S*)-reticuline occurs, followed by *ortho–para*′ benzylic coupling to afford salutaridine. Stereospecific

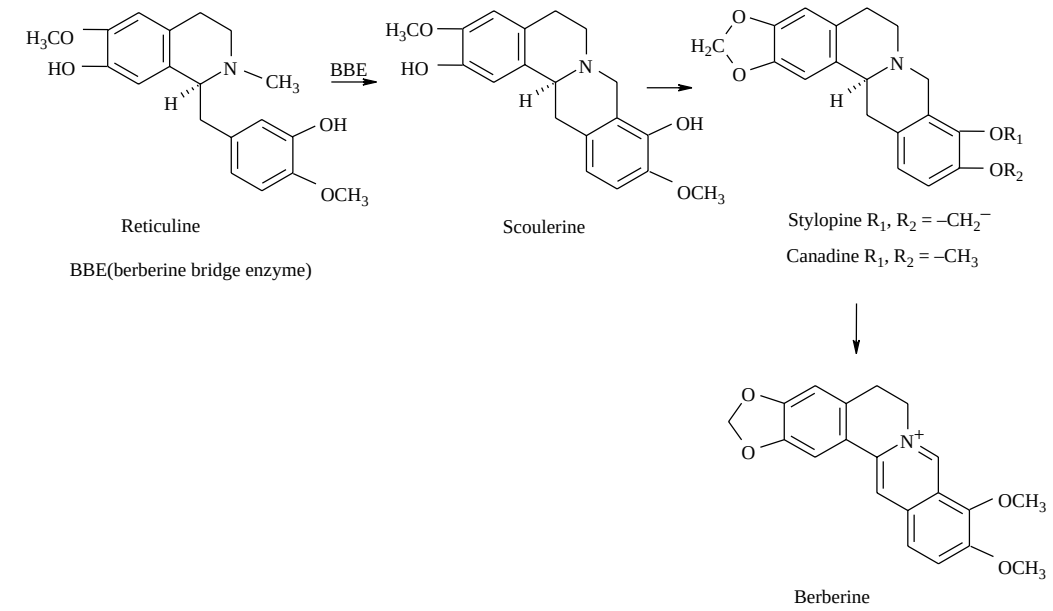


Fig. 28 The biosynthesis of protoberberine alkaloids.

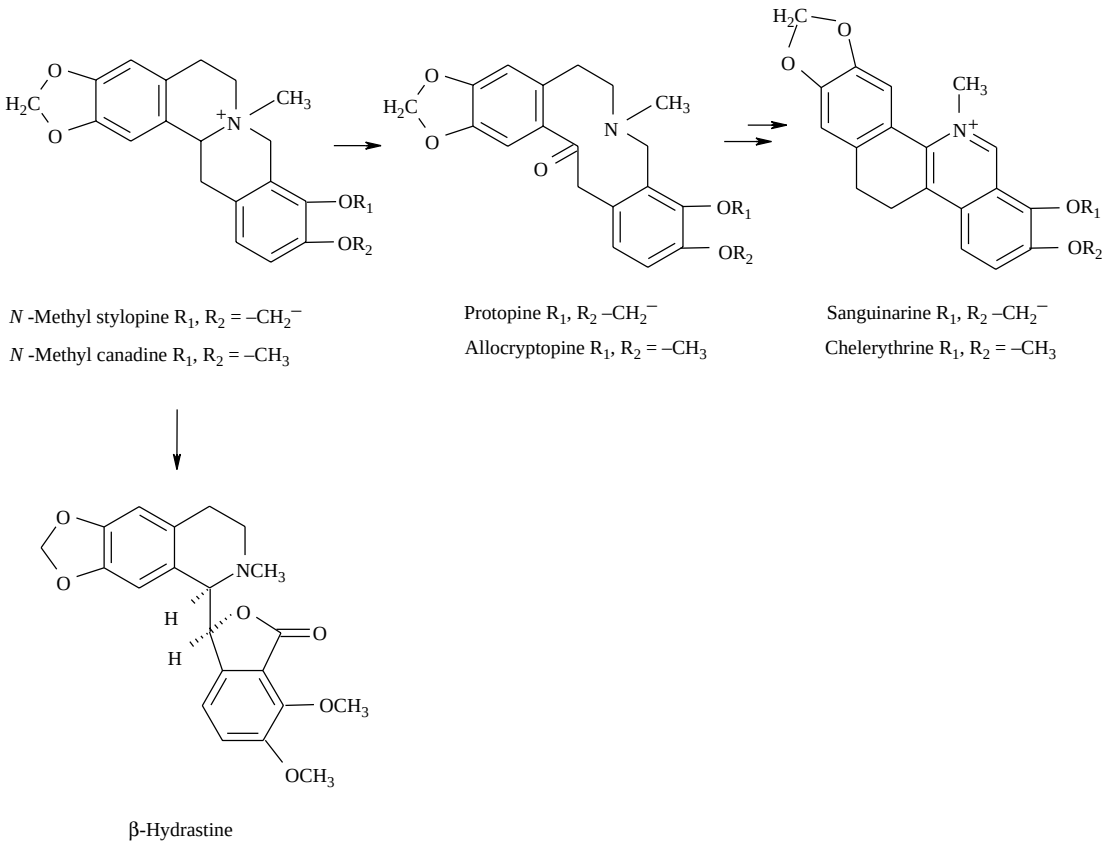


Fig. 29 The biosynthesis of β-hydrastine and sanguinarine.

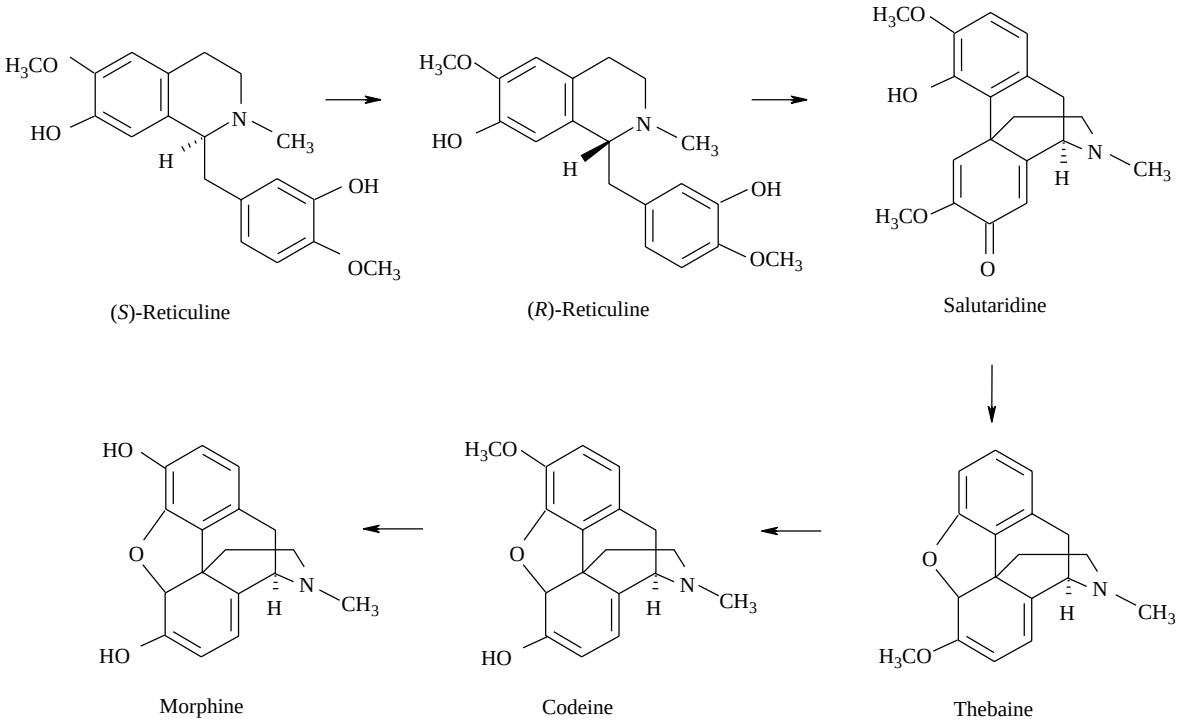


Fig. 30 The biosynthesis of morphine.

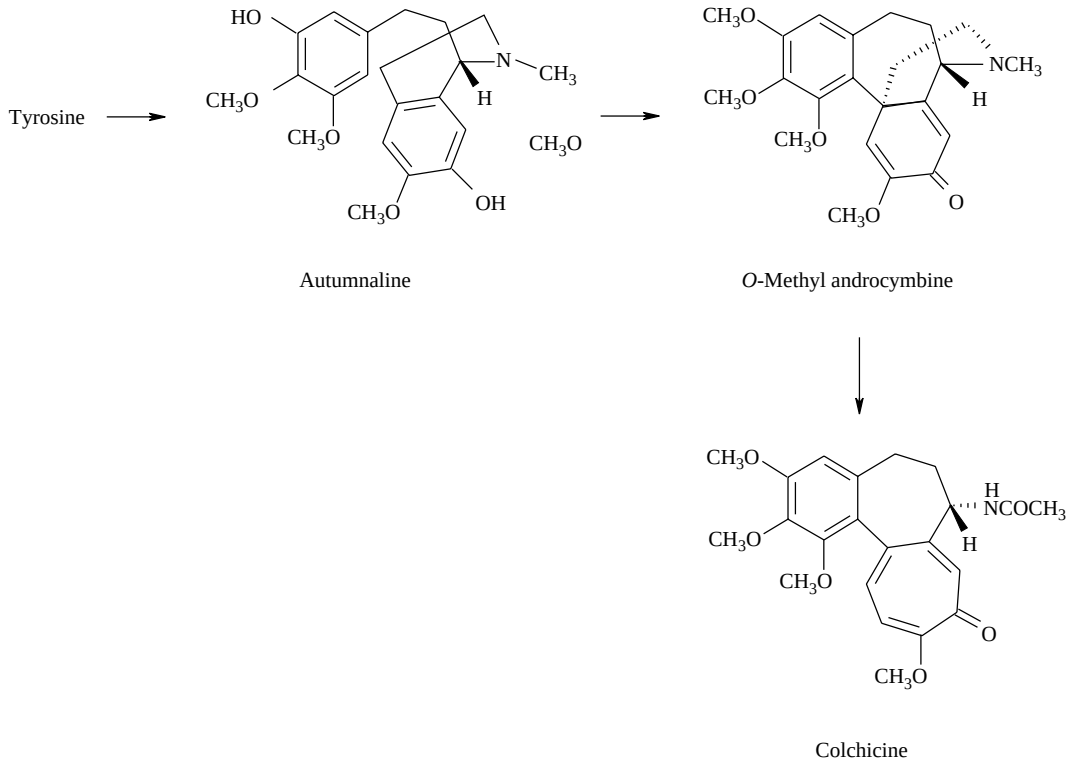


Fig. 31 The biosynthesis of colchicine.

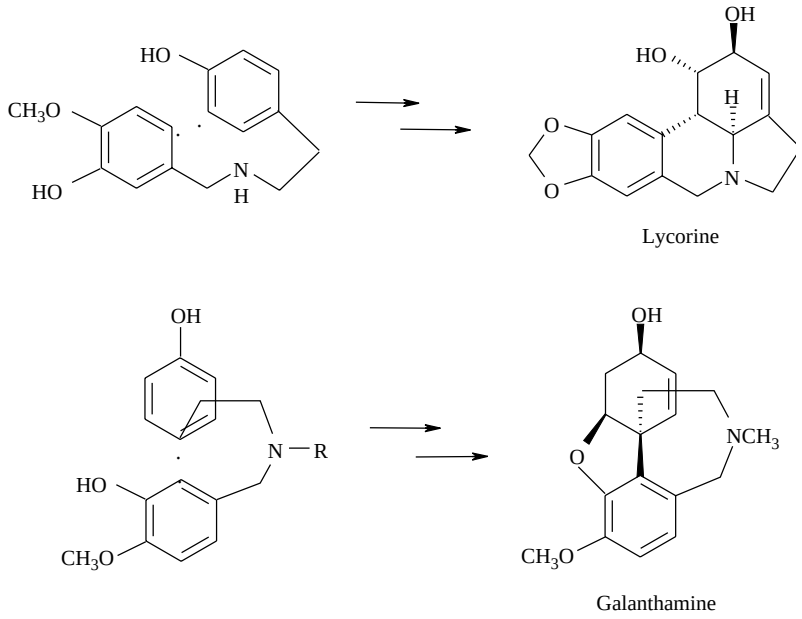


Fig. 32 The biosynthesis of some Amaryllidaceae alkaloids.

reduction and cyclization-elimination affords the 4,5-Ether bridge and thebaine. The dominant pathway from this point involves neopinone, codeinone, codeine, and morphine. Again, most of the enzymes in this sequence were isolated and characterized by Zenk's group (Fig. 30).

Morphine binds with very high affinity to several receptors in the CNS and is a potent analgesic and central depressant. It is also the prototype for many semisynthetic derivatives (e.g., naloxone, oxycodone, ethorphine, nalbuphine, and buprenophine) with various degrees of analgesic and narcotic properties. Heroin is diacetyl morphine.

Colchicine is a phenethylisoquinoline alkaloid from the autumn crocus,*Colchicum autumnale* (Liliaceae), a plant used since at least the fifth century to treat gout. *Gloriosa superba* (Liliaceae) is an alternative source. Colchicine inhibits microtubule formation at a specific site, and at a dose of 10 mg, it causes fatal respiratory arrest and renal insufficiency. It is used for the prevention and treatment of gout. The biosynthesis remains unclear; autumnaline has been proposed as an intermediate which undergoes *para–para′* coupling to afford *O*-methyl androcymbine. Hydroxylation, cyclopropane ring formation, and ring expansion affords colchicine (Fig. 31).

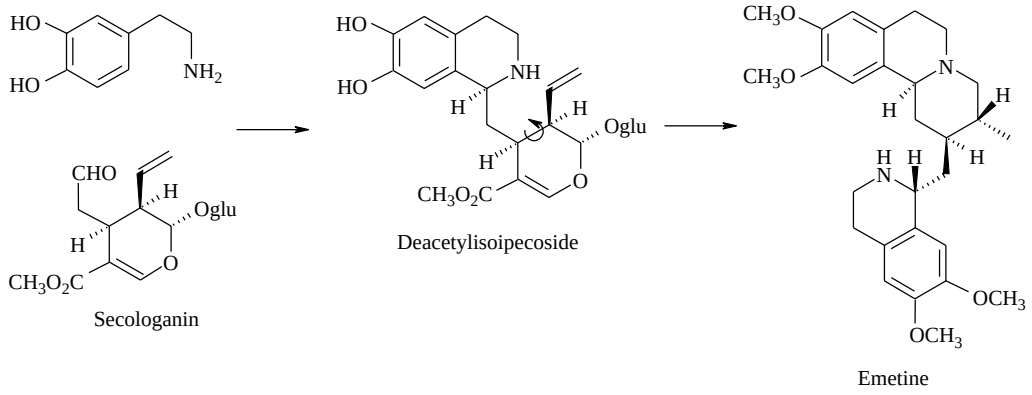


Fig. 33 The biosynthesis of emetine.

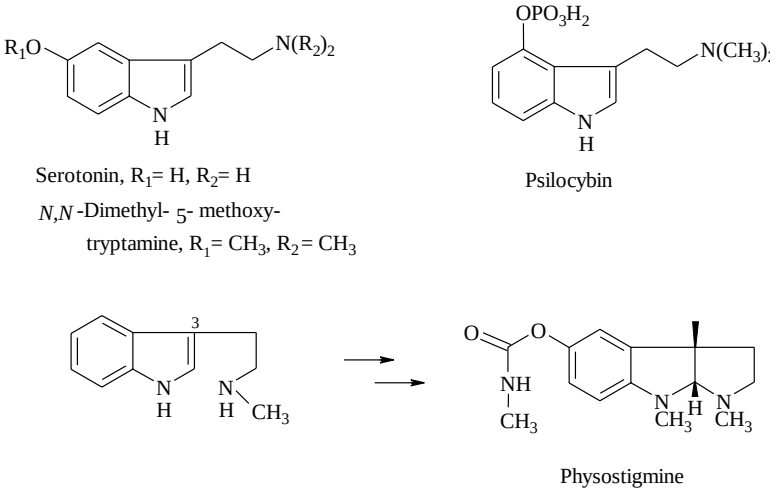


Fig. 34 Simple alkaloids derived from tryptophan.

The Amaryllidaceae alkaloids (e.g., lycorine) are derived from the phenol oxidative coupling of a $C_6C_2NC_6C_1$ unit. One unit (C_6C_2N) is derived from tyrosine, whereas the other (C_6C_1) is projected to be derived from phenylalanine, followed by oxidative deamination, hydroxylation, and cleavage of a two-carbon

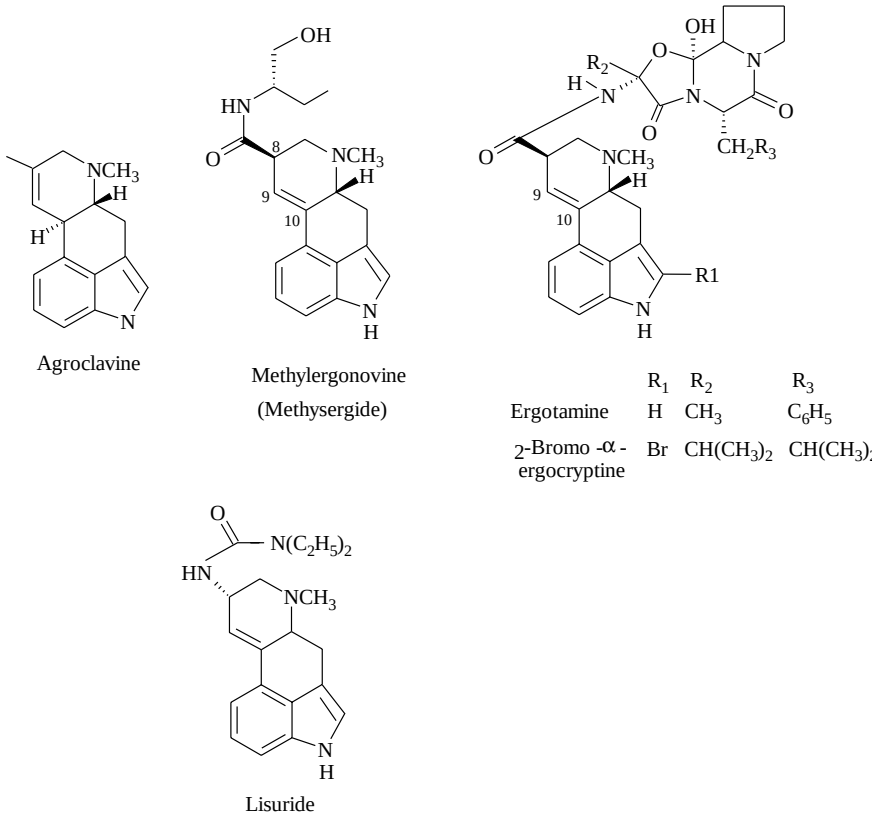


Fig. 35 Representative ergot alkaloids.

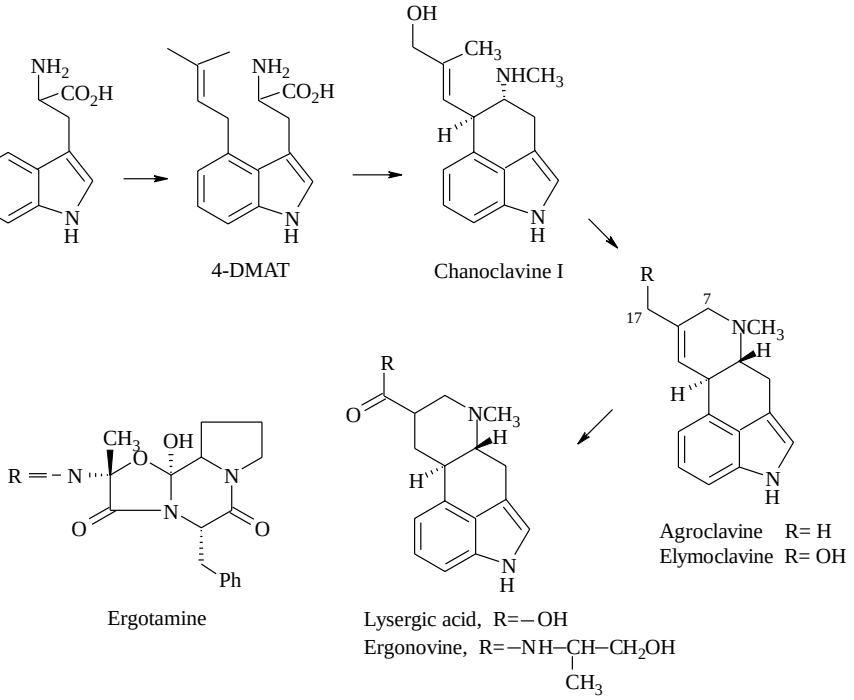


Fig. 36 The biosynthesis of the ergot alkaloids.

unit to afford a dihydroxylated benzaldehyde. *Para–ortho'* coupling leads to lycorine, whereas *para–para'* coupling affords galanthamine, of interest as a cholinesterase inhibitor (Fig. 32).

The monoterpene isoquinoline alkaloids are constituents of the genus *Cephaelis* and selected other Rubiaceae species. *C. ipecacuanha* (ipecac) is a powerful emetic whose active principle is emetine, derived through the condensation of dopamine and secologanin (Fig. 33). Emetine is also a powerful amebicide, antiviral, and inhibitor of protein synthesis. It is now largely replaced by synthetic dehydroemetine.

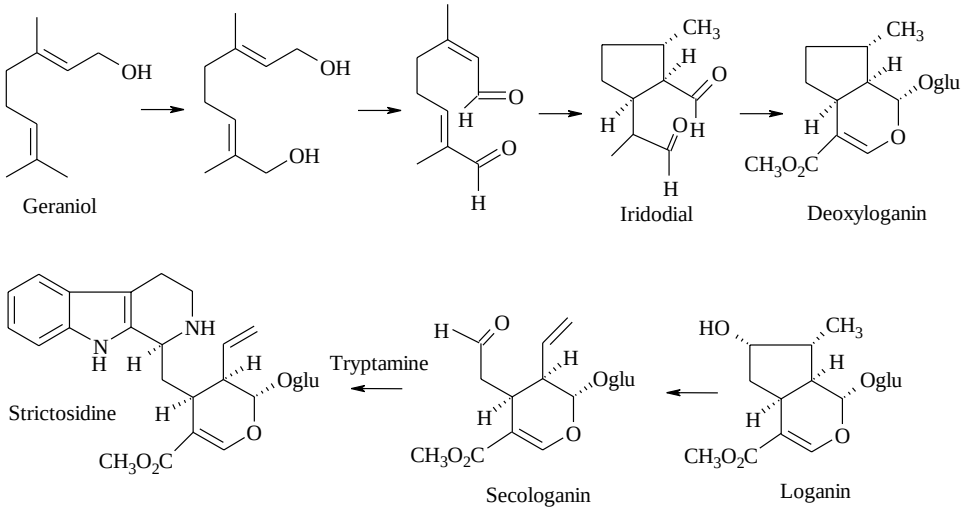
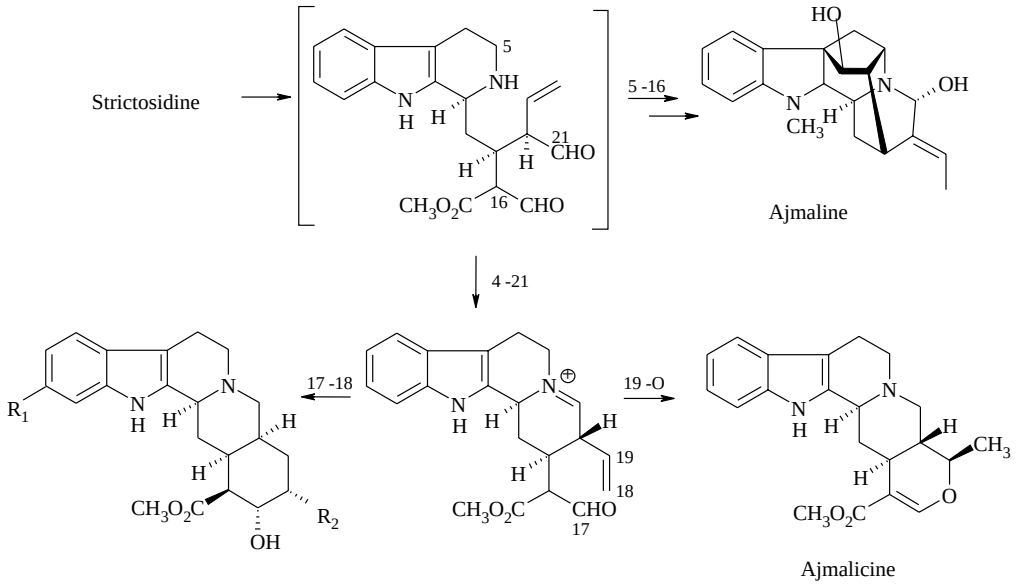


Fig. 37 The biosynthesis of strictosidine.



	R_1	R_2
α -Yohimbine	H	H
Reserpine	OCH_3	O(3,4,5 -trimethoxy benzoyl)
Rescinnamine	OCH_3	O cinnamoyl

Fig. 38 The biosynthesis of ajmaline, ajmalicine, α-yohimbine, reserpine, and rescinnamine.

ALKALOIDS DERIVED FROM TRYPTOPHAN

Numerous clinically important alkaloids are also derived from-tryptophan and are frequently referred to as "indole alkaloids." They range in molecular complexity from the mammalian hormone serotonin to the complex bisindolic anticancer alkaloid vincristine. In addition, a number of indole alkaloids, particularly those in the carbazole series, are found in peppers (*Murraya* sp.), and simple derivatives of tryptamine (e.g., *N,N*-dimethyl-5-methoxy-tryptamine and psilocybin) are found in several sources (e.g., snuffs, mushrooms, and toad skins) with attributed hallucinogenic properties.

Physostigmine, under investigation for potential use in Alzheimer's disease, is possibly formed from N_b-methyltryptamine through a radical mechanism involving C-3 methylation and concomitant C-2 cyclization, followed by N_b-methylation (Fig. 34).

The powerful biological effects of ergot have been known for over 1000 years. Ergot is a fungus (*Claviceps purpurea* and species) that grows parasitically on rye and some other grains, and it produces three types of ergot alkaloids—the clavines, the simple lysergic acid-derived, and the peptide lysergic acid-derived alkaloids. The alkaloids find therapeutic use as agents for postpartum hemorrhage (methylergonovine) and as vasoconstrictors and vasoregulators (ergotamine and 9,10-dihydroergotamine) for migraine headaches. More elaborate synthetic derivatives are also available, including lisuride (for Parkinsonism) and 2-bromo-α-ergocryptine (for prolactin-secreting adenomas and Parkinsonism; Fig. 35).

The biosynthetic pathway to the ergoline nucleus proceeds through 4-dimethylallyl tryptophan (4-DMAT), chanoclavine-I, agroclavine, and lysergic acid. Two *cis, trans* isomerizations occur: one before chanoclavine-I and the other before agroclavine, as shown by experiments with [2-^{14}C]-mevalonic acid and [Z-CH_3]-4-DMAT (Fig. 36). The peptide unit is derived from a combination of three amino acids, one of which is always proline. Several genera in the plant family Convolvulaceae (*Rivea, Ipomoea*, etc.) also produce ergot alkaloids.

The largest group of alkaloids is the monoterpene indole alkaloids, distributed in the Apocynaceae (mutual exclusion with cardenolides) and in the Rubiaceae and Loganiaceae. The molecular acrobatics of the various systems derived from deglucosylation of the primordial alkaloid strictosidine accounts for this stunning structural diversity.

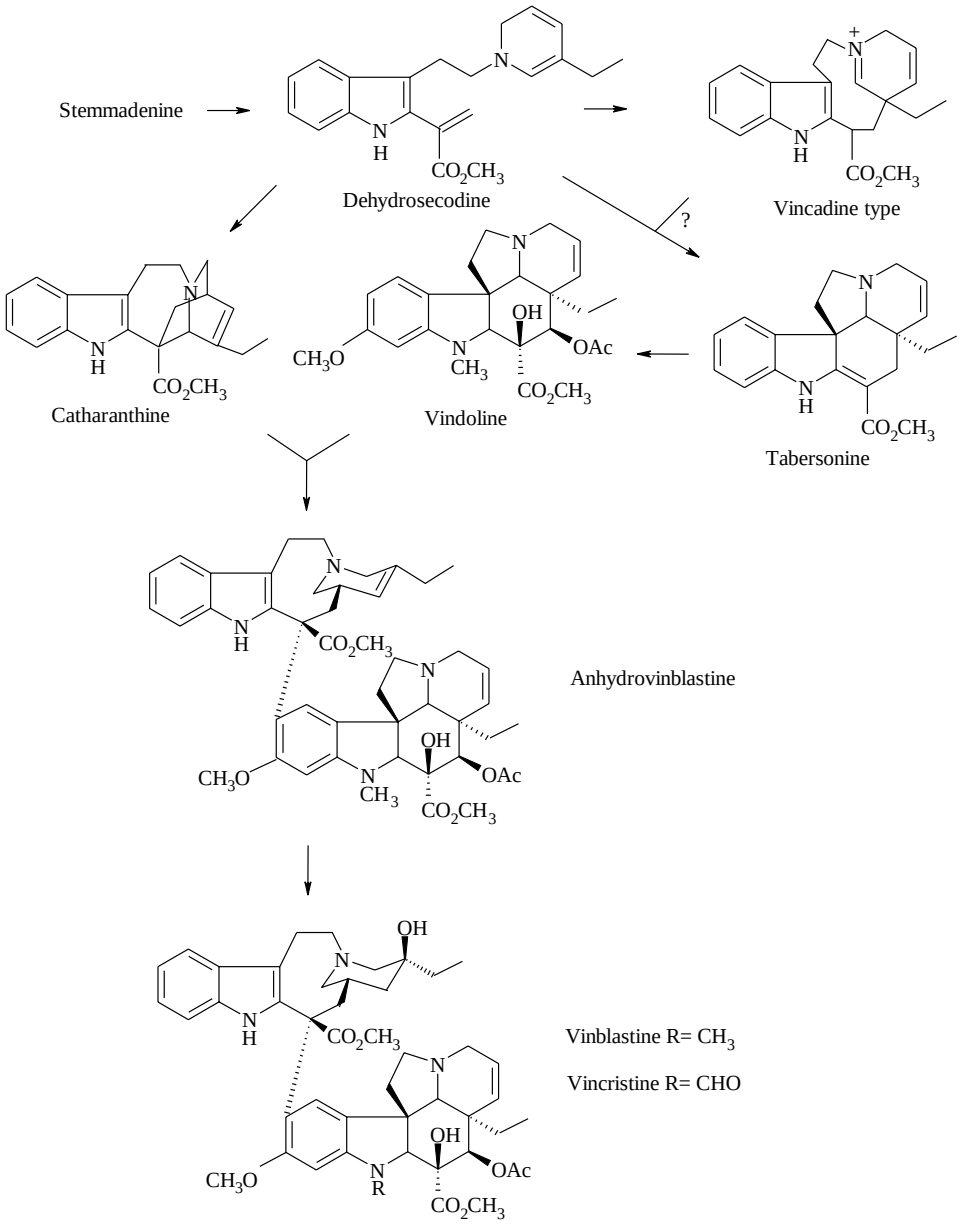


Fig. 39 The biosynthesis of vinblastine.

Strictosidine is produced, stereospecifically, from tryptamine and secologanin by strictosidine synthase, isolated from several species producing monoterpene indole alkaloids. The enzyme was cloned and can be expressed in large quantity (Fig. 37).

After deglucosylation, the pathway proceeds through a 4,21-Dehydrogeissoschizine derivative to ajmalicine (an α-Blocking spasmolytic agent, used for tinnitus and cranial trauma with an ergot derivative). If cyclization occurs between C-17 and C-18, the yohimbine nucleus is produced, whose derivatives include the *Rauvolfia* alkaloids reserpine and rescinnamine (antihypertensive activity). Ajmaline, formerly used as an antiarrythmic, also occurs in *Rauvolfia* species, and several of the enzymes in the pathway have been isolated. Recent considerations suggest that the C-16–C-5 bond may be formed before the N-4–C-21 bond (Fig. 38).

The subsequent steps from geissoschizine to form the *Strychnos* alkaloids, the secodines, the *Aspidosperma* alkaloids and the iboga alkaloids remain speculative, based

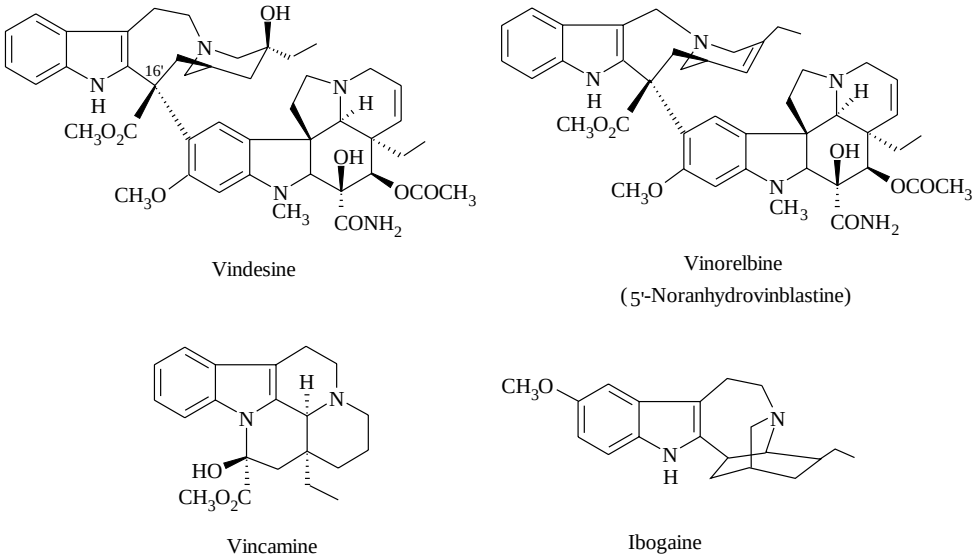


Fig. 40 Representative advanced indole alkaloids.

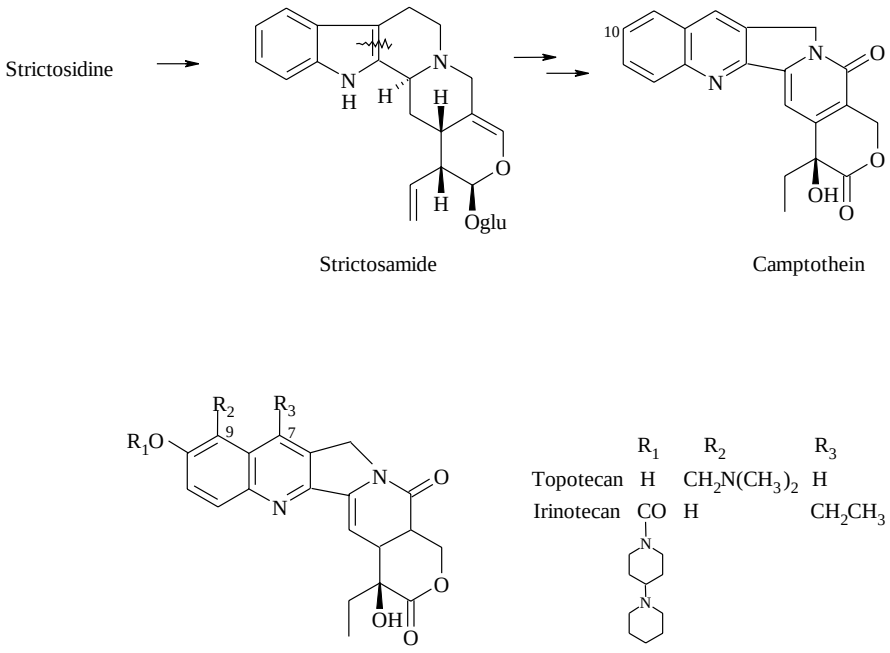


Fig. 41 The biosynthesis of camptothecin.

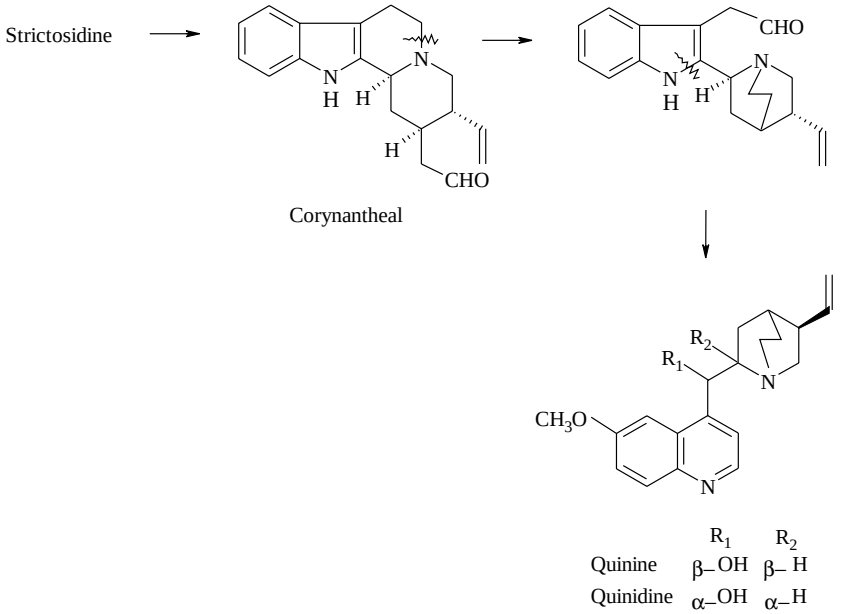


Fig. 42 The biosynthesis of quinine and quinidine.

on low levels of incorporation of early precursors or alkaloid time course studies. Joining C-2 and C-16 while moving C-3 to C-7, yields the strychnan skeleton of strychnine (lethal dose 0.2 mg/kg), still used as a rodenticide in some countries.

If the C-15,C-16 bond is oxidatively cleaved, the secodine skeleton results (the proposed progenitor of the *Aspidosperma* and the iboga systems) through alternative Diels–Alder type cyclizations to afford tabersonine and catharanthine. The bisindole alkaloids of *Catharanthus roseus* reflect the union of vindoline and catharanthine to afford anhydrovinblastine; modification affords the clinically significant alkaloids, vinblastine (VLB) and vincristine (VCR; Fig. 39). The alkaloids, particularly VCR, are important as anticancer agents and have led to the development of the semisynthetic derivatives vindesine and vinorelbine (Fig. 40). Synthetic approaches are available to join the monomeric precursors. The enzymatically controlled sequence of reactions from tabersonine to vindoline has been elucidated.

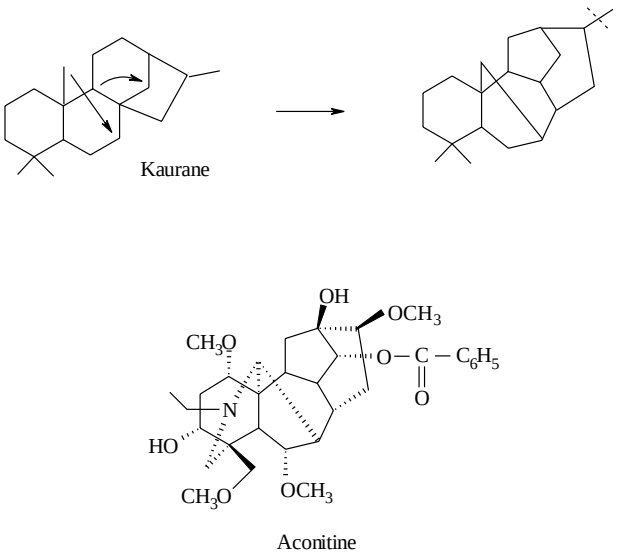


Fig. 43 The biogenesis of aconitine.

Through a biomimetic approach, tabersonine is also the semisynthetic precursor of vincamine, a Eburna alkaloid isolated from *Vinca minor*, and is used for cerebral insufficiency in Europe. *Tabernanthe iboga* has a long history of use as a stimulant in tropical Africa; its main active principle is ibogaine, a controlled substance in many countries (Fig. 40). It is being actively investigated in the United States for its potential to induce opium addiction withdrawal.

Camptothecin, a quinoline alkaloid from *Camptotheca acuminata* (Nyssaceae), is derived from strictosidine through strictosamide (Fig. 41). Originally isolated in 1966, it biologically inhibits topoisomerase I, and in 1996, two derivatives, topotecan and irinotecan, were approved for the treatment of ovarian cancer and

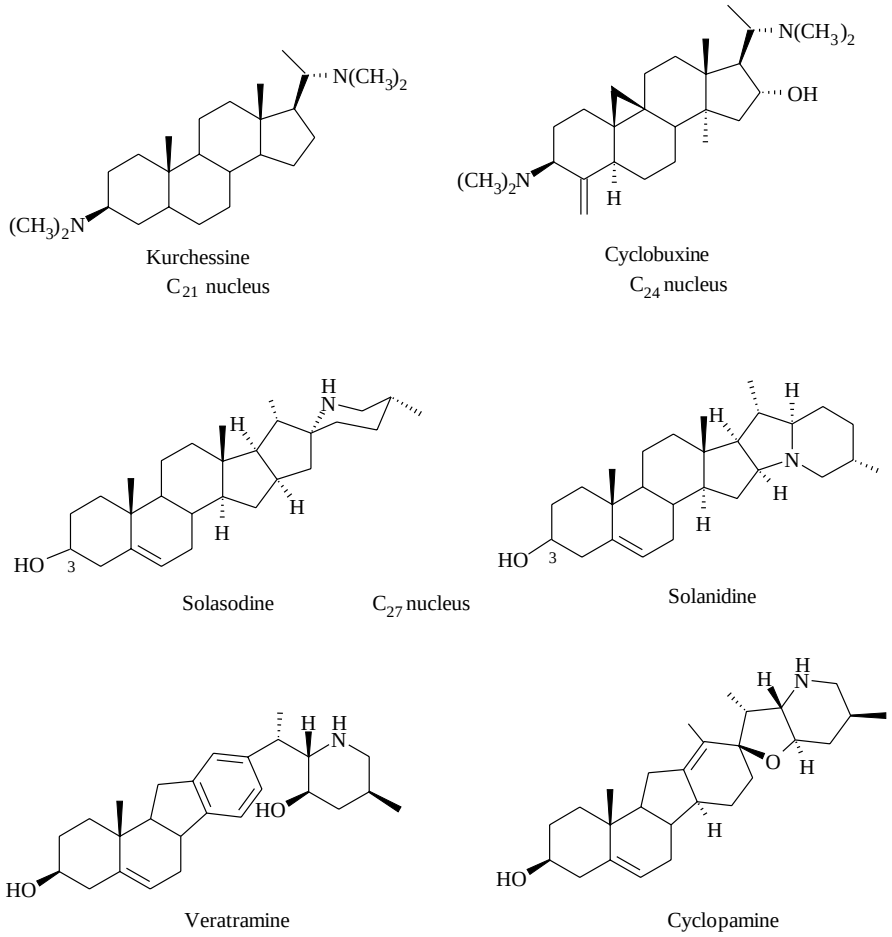


Fig. 44 Representative steroid alkaloids.

colon cancer, respectively. Other derivatives are in clinical trial.

Cinchona species (Rubiaceae) are sources of quinine and quinidine, containing a quinoline nucleus and derived through the extensive elaboration of strictosidine (Fig. 42). The intriguing history of the antimalarial quinine and its role in world politics over the past 350 years are legendary. It is frequently the only antimalarial drug to which patients are not resistant. Its widest use, however, is in the beverage industry in tonic water. Quinidine, an isomer of quinine, is used to treat cardiac arrythmias.

ALKALOIDS BASED ON A TERPENE SKELETON

Although a variety of monoterpene alkaloids and some sesquiterpene alkaloids are known, they are of little biological interest. By contrast, the diterpene alkaloids of the Ranuculaceae, for example from *Aconitum* and *Delphinium* species, have profound biological effects. The principal alkaloids of interest are those related to aconitine, acting by exciting and paralyzing peripheral nerve endings. The plants are some of the most toxic known, with merely 10 g of aconite root being lethal. Detoxified root preparations are used as drugs in several major systems of traditional medicine. Formation of the acontine nucleus is thought to occur through a rearrangement of the kaurane skeleton (Fig. 43).

The steroidal alkaloids have a nucleus based on 21, 24, or 27 carbon atoms (Fig. 44). The C_{21} alkaloids are pregnane-derived with nitrogen inserted at C-3, at C-20, or at both positions. They are characteristic of the Apocynaceae (*Funtumia* and *Holarrhena* species) and the Buxaceae (*Buxus* species). The Buxaceae also produces C_{24} alkaloids based on the cycloartane skeleton.

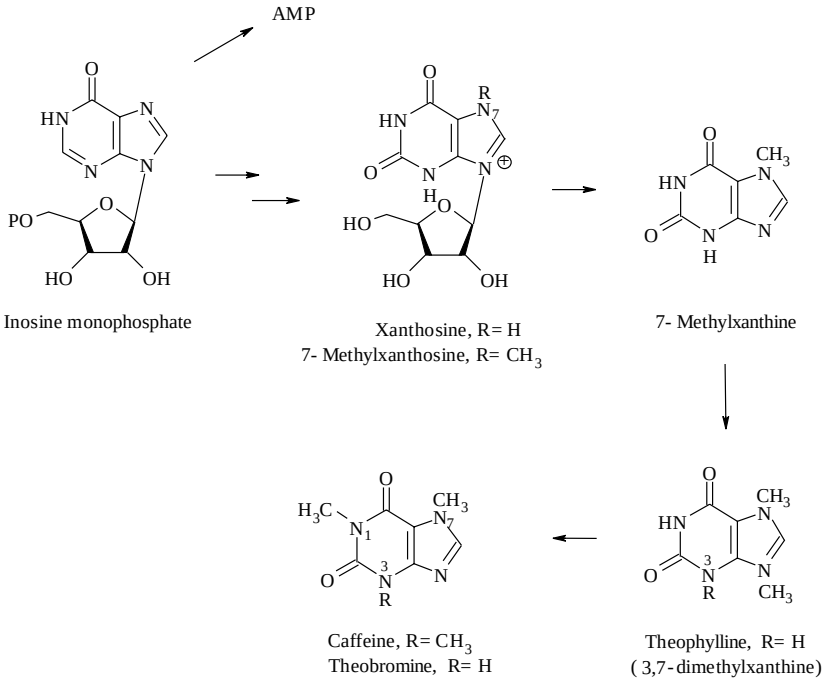


Fig. 45 The biosynthesis of purine alkaloids.

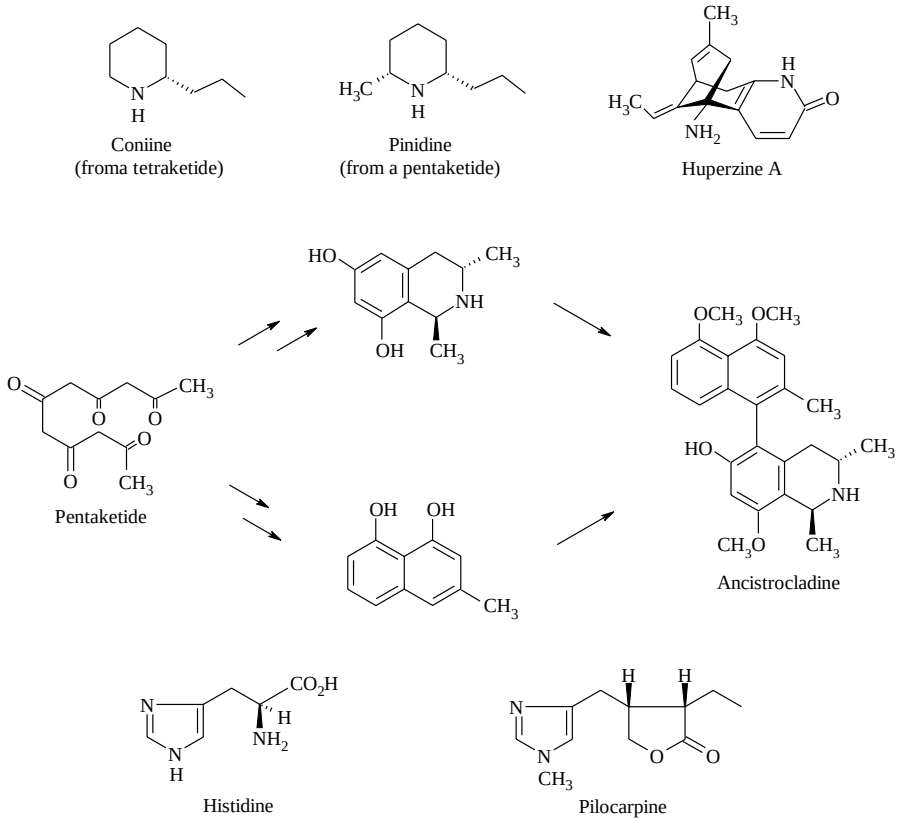


Fig. 46 Some alkaloids derived from acetate and histidine.

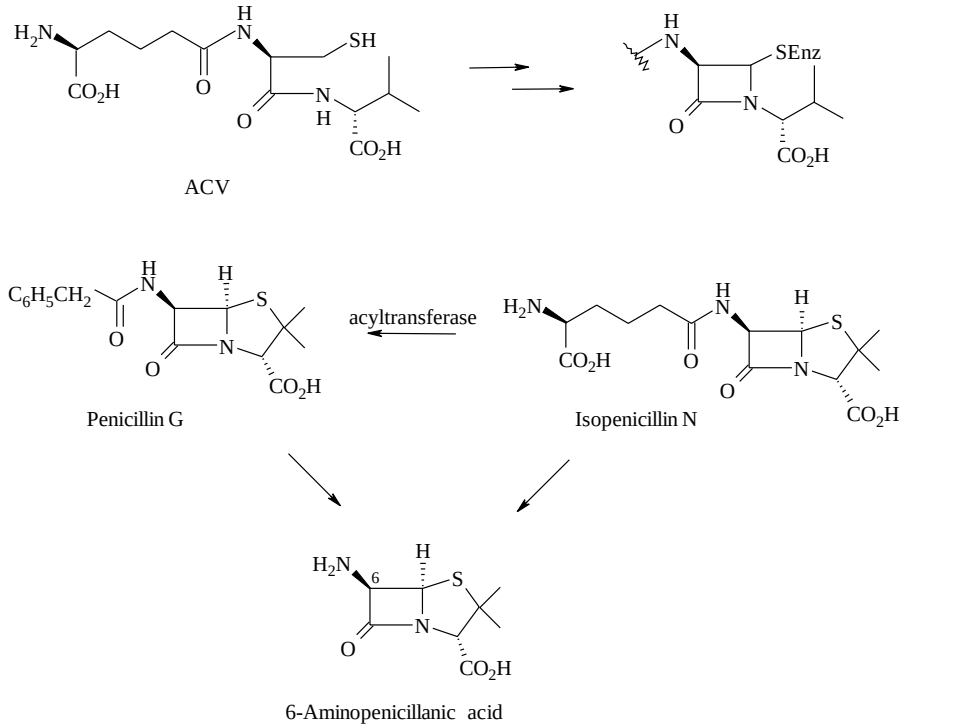


Fig. 47 The biosynthesis of penicillin G.

The most interesting alkaloids are those in the Solanaceae and the Liliaceae. These are C_{27} alkaloids, and examples include solasodine and solanidine; many derivatives are glycosylated. The alkaloids from the Liliaceae, such as veratramine of the white hellebore (*Veratrum album*), were formerly used for cardiac insufficiency. Other alkaloids, for example cyclopamine, are potent teratogens. Biogenetically, they are derived through nitrogen insertion at C-22, followed by a rearrangement generating a C-*nor*-D-*homo* steroid nucleus (Fig. 44). They are not used

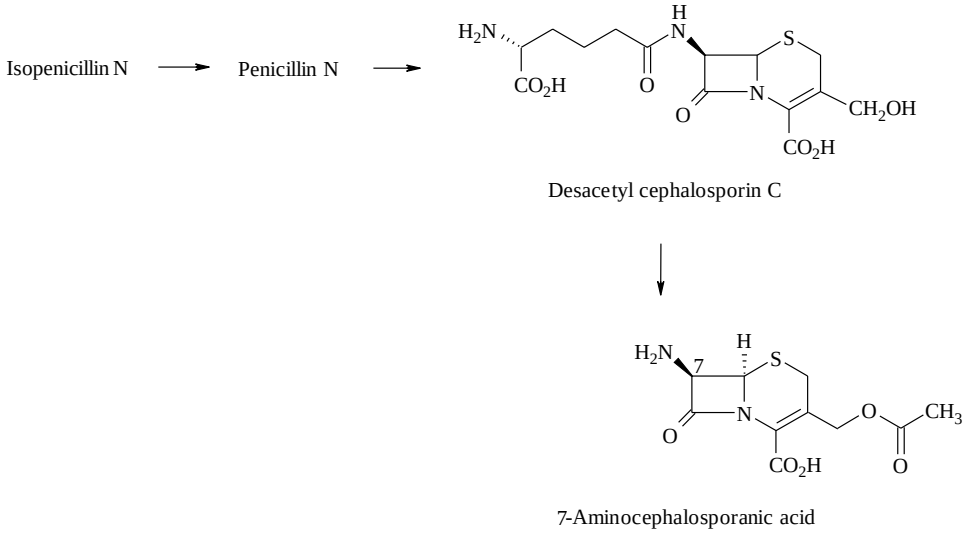


Fig. 48 The biosynthesis of desacetylcephalosporin C.

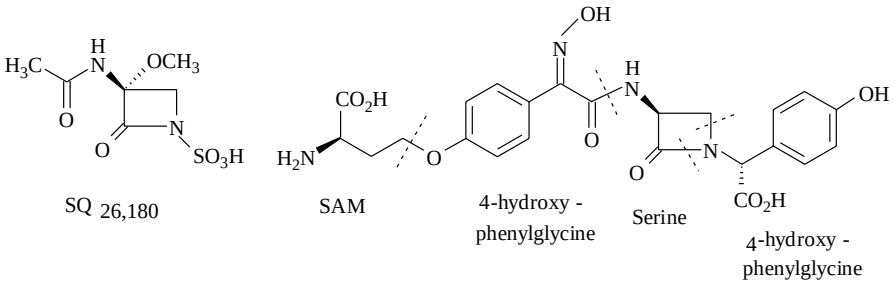


Fig. 49 The biogenesis of nocardicin A.

therapeutically, but the glycoalkaloids of *Solanum tuberosum* (potato) are very toxic and are not destroyed in cooking.

ALKALOIDS DERIVED FROM A NUCLEOTIDE PRECURSOR

Caffeine is one of most widely consumed alkaloids on a daily basis. As well as being a significant constituent of coffee (*Caffea arabica*) and tea (*Camellia sinensis*), caffeine is also present in kola (*Kola* species), guarana (*Paullinia cupana*), and maté (*Ilex paraguariensis*). All of these species are used in various parts of the world to produce beverages that reduce fatigue.

Although some steps remain to be fully elucidated, caffeine is probably derived though a pathway beginning with inosine 5′-monophosphate and proceeds through xanthosine, 7-methylxanthosine, 7-methylxanthine, 3,7-dimethylxanthine (theobromine) to caffeine (Fig. 45). The final methyltransferase has been characterized in coffee and tea, whereas the methylation of xanthosine has only been studied in tea.

Caffeine is noted for its ability to stimulate the CNS and also has positive inotropic and mild diuretic activity. Theophylline (1,7-Dimethylxanthine) is noted for its smooth muscle relaxant activity and its use for chronic asthma.

ALKALOIDS DERIVED FROM OTHER PRECURSORS

Acetate is also a precursor of several groups of alkaloids in the form of a polyketide chain that interacts with an unknown nitrogen source (as in the terpene alkaloids). Examples of acetate-derived alkaloids are coniine—the toxic principle of *Conium maculatum*, pinidine—from several *Pinus* species, and the naphthylisoquinoline alkaloids (e.g., ancistrocladine)—showing antimalarial and anti-HIV activity. The latter alkaloids are apparently derived from the oxidative coupling of two pentaketide units. Huperzine A, currently in clinical trials for the treatment of Alzheimer's disease and isolated from the club moss (*Serrata huperzia*), is derived from a polyacetate precursor (Fig. 46).

Histidine is a precursor of a very limited number of alkaloids. The most well-known is pilocarpine from *Pilocarpus jaborandi.* The plant was formerly used as a truth serum (diaphoretic activity), and the alkaloid is used to counter the mydriatic effects of atropine.

The penicillins, from the fungus *Penicillium chrysogenum*, are the oldest and most widely used antibiotics. They are formed through stepwise build-up from a tripeptide (ACV) derived from α-Amino adipic acid, cysteine, and valine. Successive oxidation steps form the β-lactam and close the thiazolidine ring to form isopenicillin N. Action of an acyltransferase then yields penicillin G (Fig. 47). Alternatively, hydrolysis of isopenicillin N (or penicillin G) yields 6-aminopenicillanic acid, a key precursor for the wide range of semisynthetic pencillins used therapeutically.

Cephalosporins are modified penicillin derivatives produced by *Cephalosporium acremonium*, wherein isomerization occurs to afford penicillin N followed by a ring expansion involving one of the methyl groups and hydroxylation to produce descetylcephalosporin C (Fig. 48). Once again, removal of the acylating side chain to afford 7-Aminocephalosporanic acid was the key to generating the diversity of available cephalosporin derivatives. The monobactams, such as SQ26,180 from *Chromobacterium violaceum*, contain a 3-Methoxy group

and an *N*-Sulphonate moiety. The carbon framework is derived from serine. More complex derivatives, such as nocardicin A, are formed through a tripeptide pathway similarly to the penicillins (Fig. 49).

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